Electronic Supplementary Information

Phosphonated Triarylmethyl Radical as Probe for Measurement of pH by EPR

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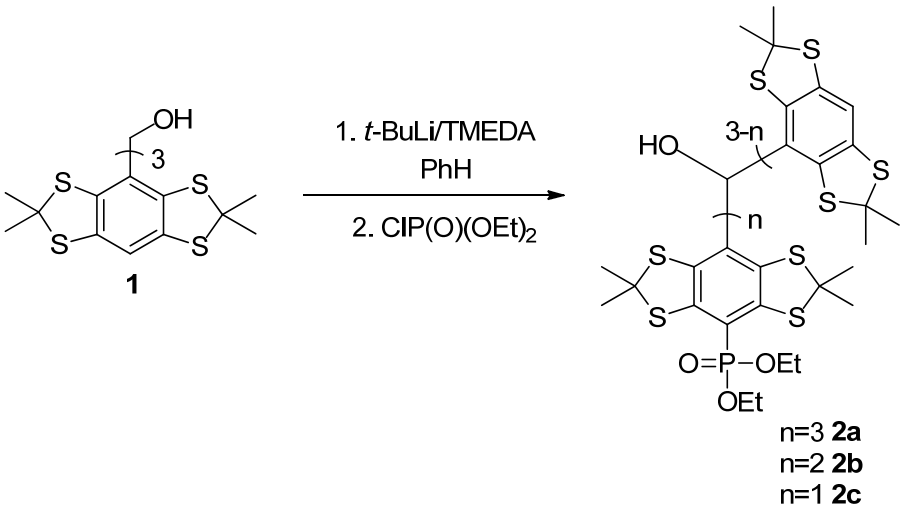
1. General information

All reactions were carried out in flame-dried glassware under argon atmosphere. Anhydrous grade solvents were used for reactions and HPLC grade for purifications. All commercially available reagents were used as received without further purification. NMR spectra were recorded on a BRUKER Avance II (^{1}H: 300 MHz, ^{13}C: 75 MHz, ^{31}P 121 MHz) and a BRUKER Avance DPX (^{1}H: 500 MHz, ^{13}C: 125 MHz). Chemical shifts (δ) are reported in parts per million (ppm), referenced to NMR solvents, chloroform-d (δ ^{1}H=7.27 ppm, ^{13}C=77.2 ppm) and to H_3PO_4 85% (δ ^{31}P=0 ppm). The following abbreviations are used: s=singlet, d=doublet, t=triplet, m=multiplet. Coupling constants (J) are reported in Hertz (Hz). Infrared (IR) spectra are recorded using a Shimadzu spectrometer FTIR-8400S; products were analyzed as thin films deposited on a Se−Zn crystal by evaporation from the solvent. High resolution mass spectrometry (HRMS) analyses were performed at the University College London. Analytical HPLC was carried out on a Waters Alliance 2690 separations module equipped with a Waters 2998 PDA detector. Semi-preparative HPLC was carried out on a Waters 600 Pump equipped with a Waters 486 UV detector, a Waters fraction collector III and a Hitachi L-7200 HPLC Autosampler. EPR spectra were recorded on an X-Band BRUKER EMX spectrometer equipped with a ER4119HS resonator. pH was measured with a inoLab pH 730 pH-meter.

2. Chemistry

2.1 Synthesis of trityl alcohols 2a, 2b and 2c



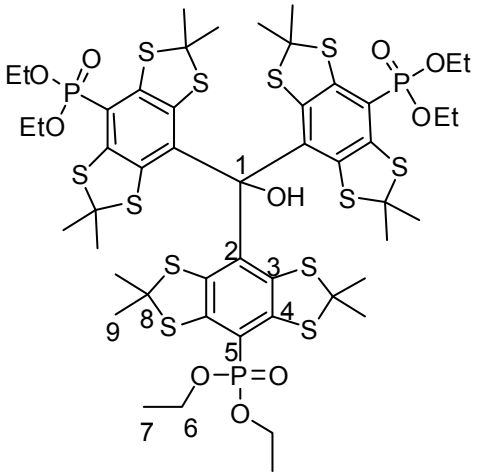
Trityl alcohol **1** (containing 1.3 eq. of benzene) (400 mg, 0.405 mmol, 1eq.) was dissolved in dry benzene (5 mL), TMEDA (611 µL, 4.052 mmol, 10 eq.) was added. The solution was cooled at 0°C and t-BuLi 1.7 M in pentane (2.38 mL, 4.052 mmol, 10 eq.) was added dropwise under stirring. The mixture was stirred at 0°C for 2h then added to a solution of diethyl chlorophosphate (2.93 mL, 20.263 mmol, 50 eq.) in dry benzene (5 mL) at 0°C. The mixture was stirred 30 min at 0°C and 1h30 at room temperature. The reaction was quenched with $KH_2PO_{4sat.}$ (10 mL) and the layers were separated, the organic layer was washed with $NH_4Cl_{sat.}$ (1 x 10 mL), Na_2CO_3 (1 x 10 mL), H_2O (1 x 10 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent removed under reduced pressure. The dark-orange oil was heated at 85°C under vacuum (5 x 10^{-2} mbar) to remove volatile compounds then semi-preparative HPLC afforded **2a** (178 mg, 34%), **2b** (61 mg, 13%) and **2c** (17 mg, 4%).

The conditions of purification were as follows:

Column: XBridge Prep C18 5µm 10 x 100 mm at ambient temperature equipped with a guard column XBridge Prep C18 5µm 10 x 20 mm, helium sparge 30 mL/min, UV detection at 364 nm. Gradient elution:

Time	Flow	H_2O	ACN	Curve (Waters)
0.00 min	5 mL/min	40%	60%	
1.00 min	5 mL/min	40%	60%	6 (linear)
13.00 min	5 mL/min	0%	100%	6
16.00 min	5 mL/min	0%	100%	6
16.10 min	5 mL/min	40%	60%	6

Tris(8-diethoxyphosphoryl-2,2,6,6-tetramethylbenzo[1,2-d;4,5-d']bis[1,3]dithiol-4-yl)methanol (**2a**)



Aspect: Orange solid.

Rf (SiO_2): 0.13 DCM/EtOAc (80:20) UV (254 nm).

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.33 (t, *J*=6.9 Hz, 18H, H-7), 1.59 (s, 9H, H-9), 1.67 (s, 9H, H-9'), 1.73 (s, 9H, H-9''), 1.76 (s, 9H, H-9'''), 4.05-4.24 (m, 12H, H-6), 6.51 (s, OH).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 16.4 (m, C-7), 27.1 (C-9), 29.0 (C-9'), 32.2 (C-9''), 35.1 (C-9'''), 61.1 (C-8), 61.3 (C-8'), 62.9 (m, C-6), 84.4 (C-1), 118.8 (d, *J*=184.6 Hz, C-5), 134.4 (d, *J*=2.3 Hz, C-2), 140.0 (d, *J*=14.6 Hz, C-3), 140.6 (d, *J*=14.2 Hz, C-3'), 143.1 (d, *J*=9.0 Hz, C-4), 144.6 (d, *J*=8.7 Hz, C-4').

^{31}P NMR (121 MHz, $CDCl_3$) δ (ppm): 16.3.

ATR-IR (cm^{-1}): 2978, 2908, 1452, 1364, 1294, 1252, 1211, 1018.

HRMS (TOF MS ES+) *m/z*: $[M+H]^+$ calcd for $C_{49}H_{68}O_{10}P_3S_{12}$: 1293.0674, found: 1293.0542.

RP-HPLC: RT=1.1 min, column: XBridge C18 2.5 µm 4.6 x 50mm equipped with a guard column XBridge C18 2.5 µm 4.6 x 20mm. Column temperature: 40°C, UV detection at 254 nm.

Time	Flow	H_2O	ACN	Curve (Waters)
	1.5 mL/min	20%	80%	
4.00 min	1.5 mL/min	0%	100%	6 (linear)
6.00 min	1.5 mL/min	0%	100%	6
6.10 min	1.5 mL/min	20%	80%	6

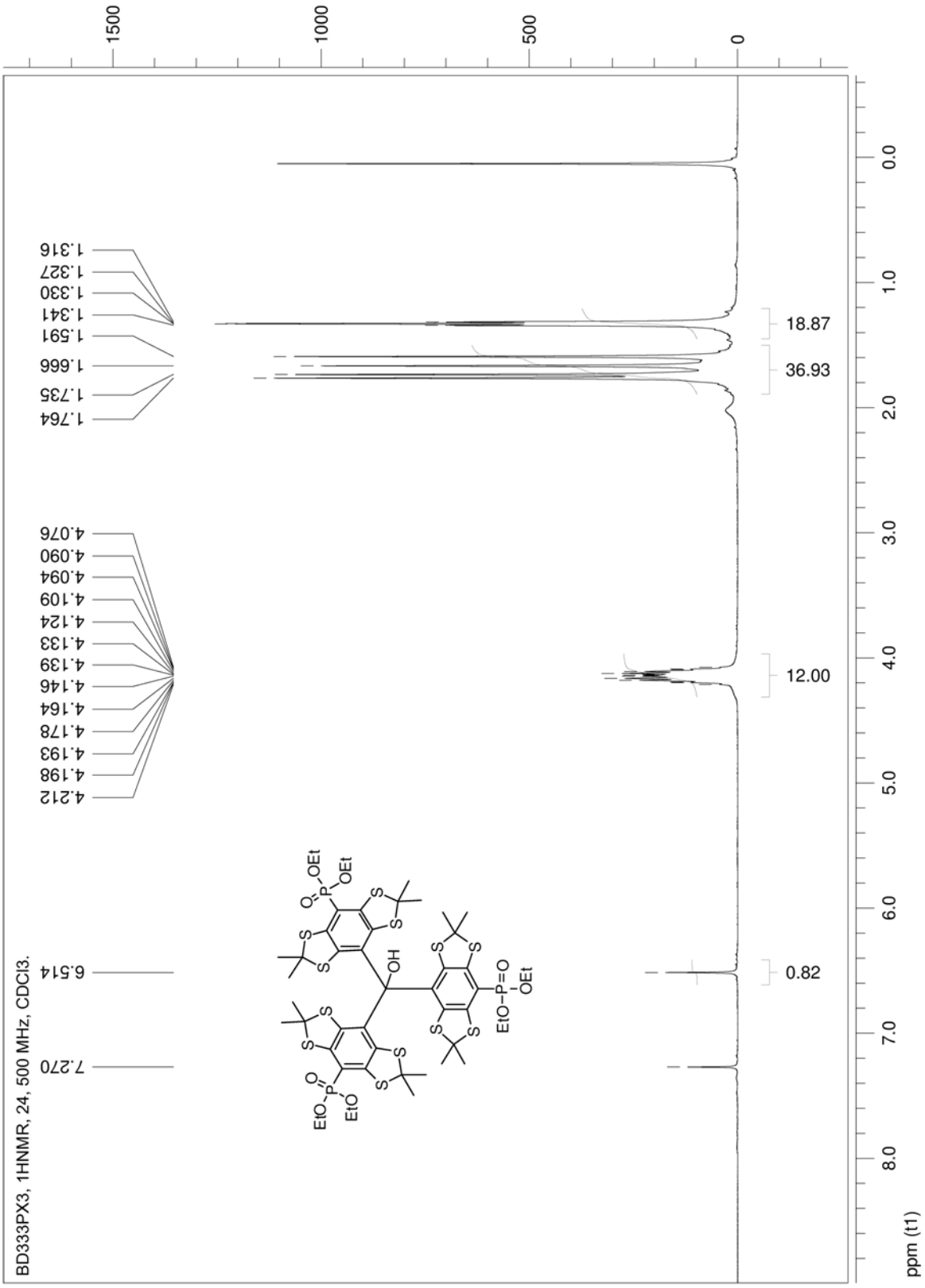


Figure S1: ^{1}H NMR 500 MHz spectrum of **2a**

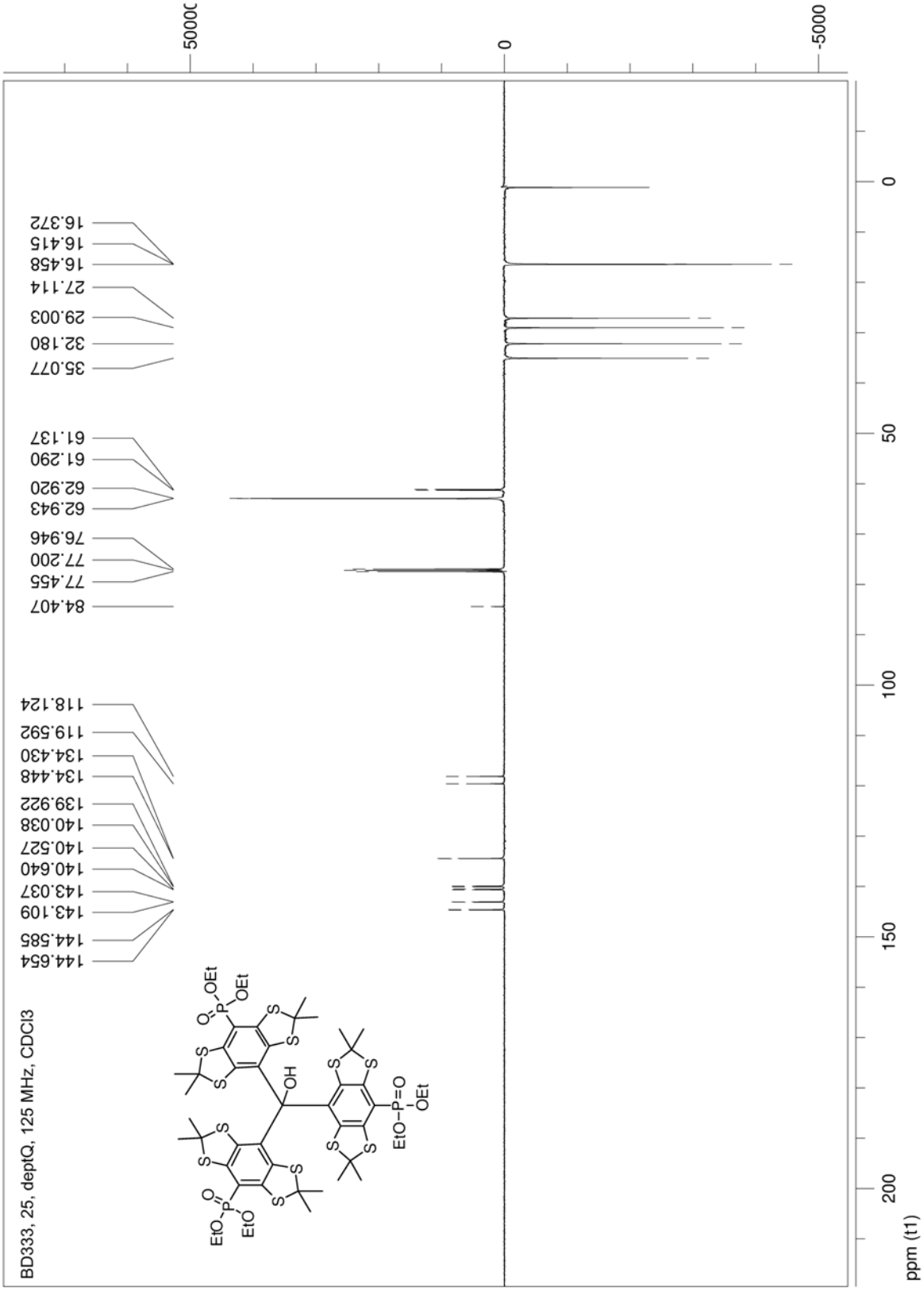


Figure S2: ^{13}C NMR 125 MHz spectrum of **2a**

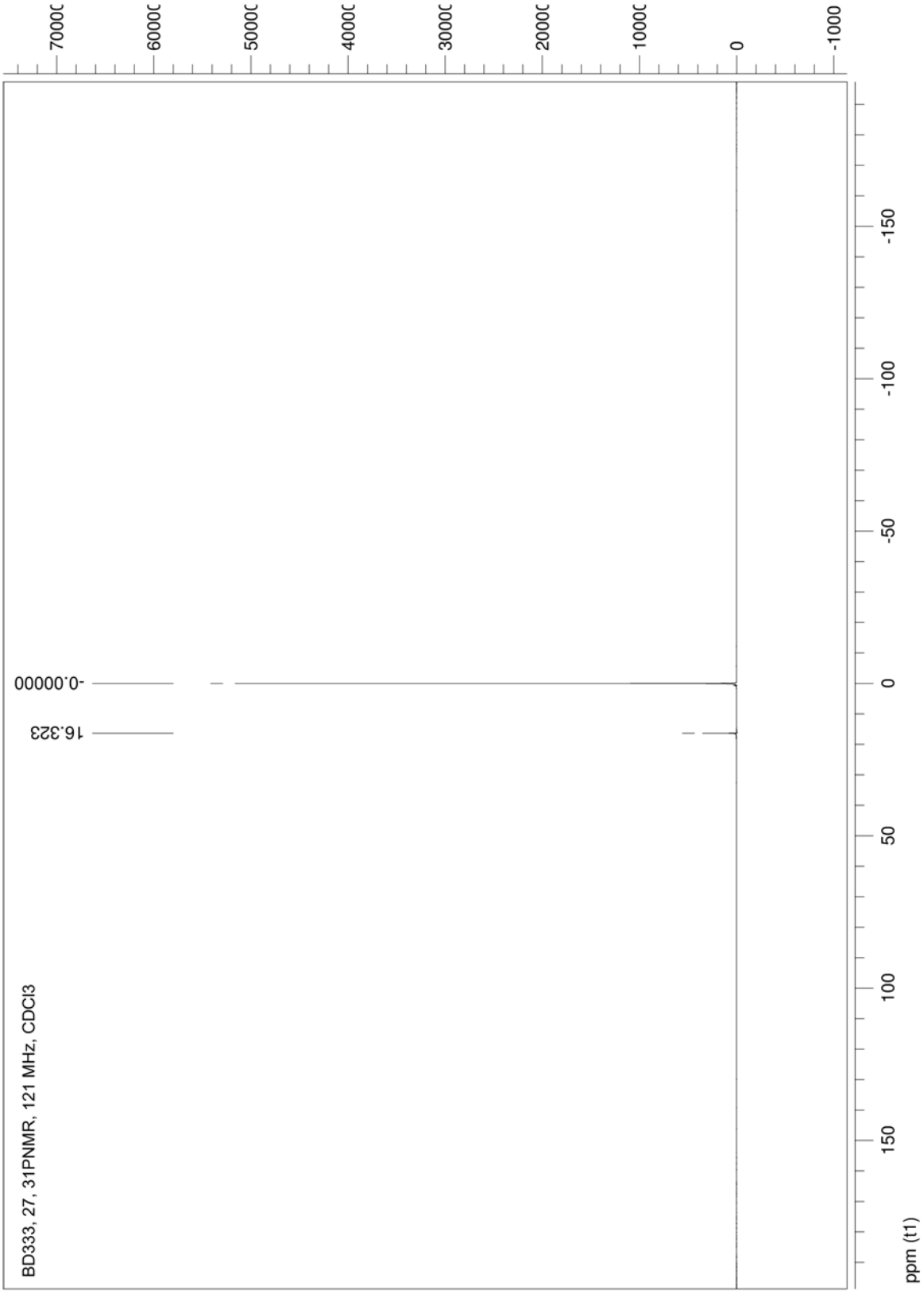


Figure S3: ^{31}P NMR 121 MHz spectrum of **2a**

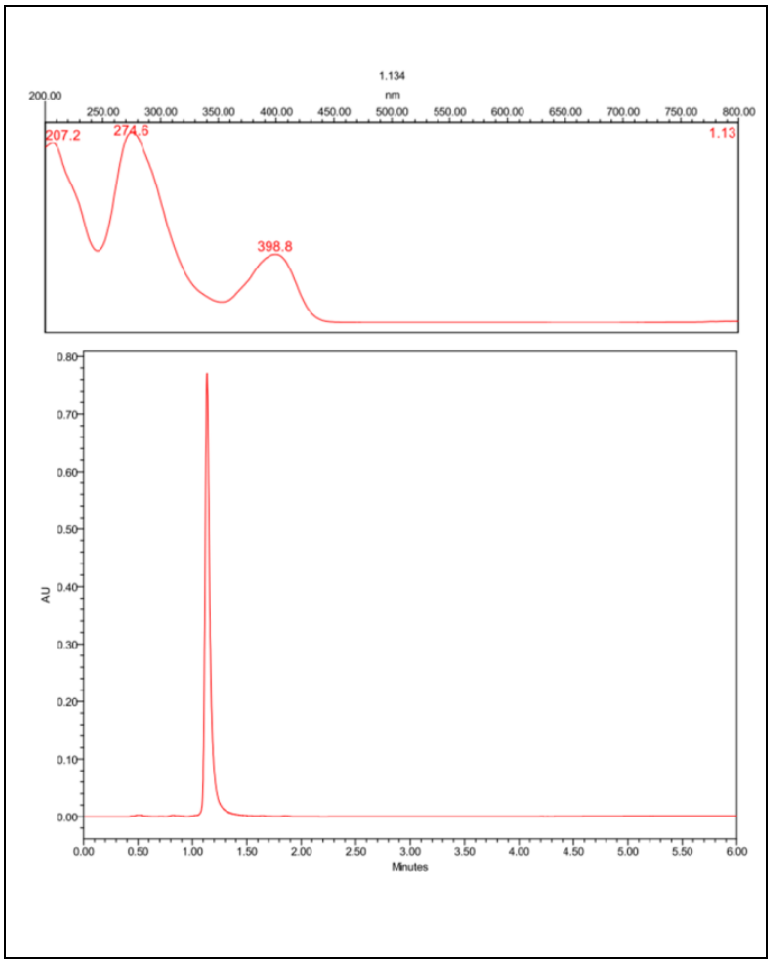


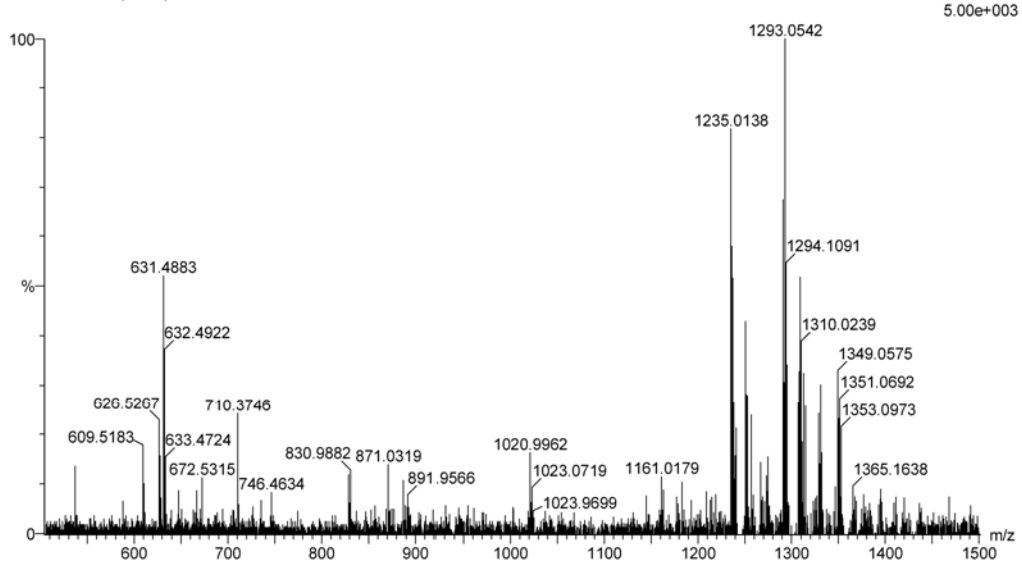
Figure S4: HPLC/UV of **2a**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 11.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
8 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 49-49 H: 4-250 O: 7-10 Na: 0-1 P: 3-3 S: 12-12

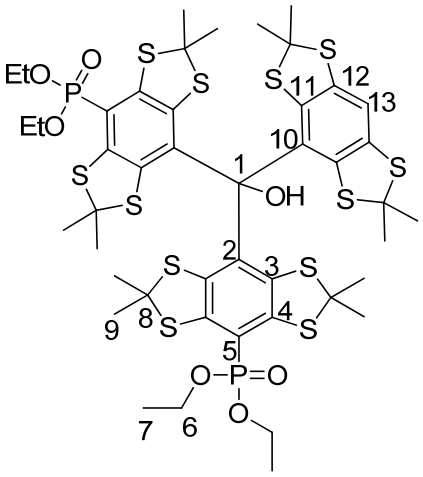
BD333PX3a 14 (0.485) 1: TOF MS ES+ 5.00e+003



Minimum:				-1.5		
Maximum:		20.0	11.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
1293.0542	1293.0674	-13.2	-10.2	17.5	543.1	C49 H68 O10 P3 S12

Figure S5: HRMS of **2a**

Bis(8-diethoxyphosphoryl)tris(2,2,6,6-tetramethylbenzo[1,2-d;4,5-d']bis[1,3]dithiol-4-yl)methanol (**2b**)



Aspect: Orange solid.

Rf (SiO_2): 0.33 DCM/EtOAc (80:20) UV (254 nm).

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.35 (t, *J*=7.1 Hz, 12H, H-7), 1.60 (s, 3H, H-9), 1.62 (s, 3H, H-9'), 1.67 (s, 3H, H-9''), 1.68 (s, 3H, H-9'''), 1.68 (s, 3H, H-9$^{4'}$), 1.72 (s, 3H, H-9$^{5'}$), 1.73 (s, 3H, H-9$^{6'}$), 1.76 (s, 3H, H-9$^{7'}$), 1.78 (s, 3H, H-9$^{8'}$), 1.79 (s, 3H, H-9$^{9'}$), 1.81 (s, 3H, H-9$^{10'}$), 1.81 (s, 3H, H-9$^{11'}$), 4.08-4.24 (m, 8H, H-6), 6.43 (s, OH), 7.20 (s, 1H, H-13).

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 16.5 (m, C-7), 27.1 (C-9), 27.4 (C-9'), 27.5 (C-9''), 28.5 (C-9$^{3'}$), 29.1 (C-9$^{4'}$), 29.8 (C-9$^{5'}$), 31.9 (C-9$^{6'}$), 32.1 (C-9$^{7'}$), 32.7 (C-9$^{8'}$), 34.9 (C-9$^{9'}$), 35.2 (C-9$^{10'}$+C-9$^{11'}$), 60.9 (C-8), 61.1 (C-8'+C-8''), 61.3 (C-8$^{3'}$), 62.9 (m, C-6), 63.7 (C-8$^{4'}$), 64.7 (C-8$^{5'}$), 84.2 (C-1), 118.6 (C-13), 118.6 (d, *J*=184.5 Hz, C-5), 118.7 (d, *J*=184.2 Hz, C-5'), 131.6 (C-ArH), 134.6 (d, *J*=2.5 Hz, C-2), 134.8 (d, *J*=2.5 Hz, C-2'), 137.6 (C-ArH), 138.1 (C-ArH), 138.5 (C-ArH), 138.9 (C-ArH), 140.2 (d, *J*=14.5 Hz, C-3), 140.3 (d, *J*=14.1 Hz, C-3'+C-3''), 141.4 (d, *J*=14.2 Hz, C-3'''), 143.0 (d, *J*=9.1 Hz, C-4), 143.1 (d, *J*=9.1 Hz, C-4'), 144.2 (d, *J*=8.7 Hz, C-4''), 144.6 (d, *J*=8.8 Hz, C-4''').

^{31}P NMR (121 MHz, $CDCl_3$) δ (ppm): 16.5, 16.6.

ATR-IR (cm^{-1}): 2976, 2921, 1452, 1365, 1296, 1249, 1211, 1018.

HRMS (TOF MS ES+) *m/z*: $[M+H]^+$ calcd for $C_{45}H_{59}O_7P_2S_{12}$: 1157.0385, found: 1157.0323.

RP-HPLC: RT=2.5 min, column: XBridge C18 2.5 µm 4.6 x 50mm equipped with a guard column XBridge C18 2.5 µm 4.6 x 20mm. Column temperature: 40°C, UV detection at 254 nm.

Time	Flow	H_2O	ACN	Curve (Waters)
	1.5 mL/min	20%	80%	
4.00 min	1.5 mL/min	0%	100%	6 (linear)
6.00 min	1.5 mL/min	0%	100%	6
6.10 min	1.5 mL/min	20%	80%	6

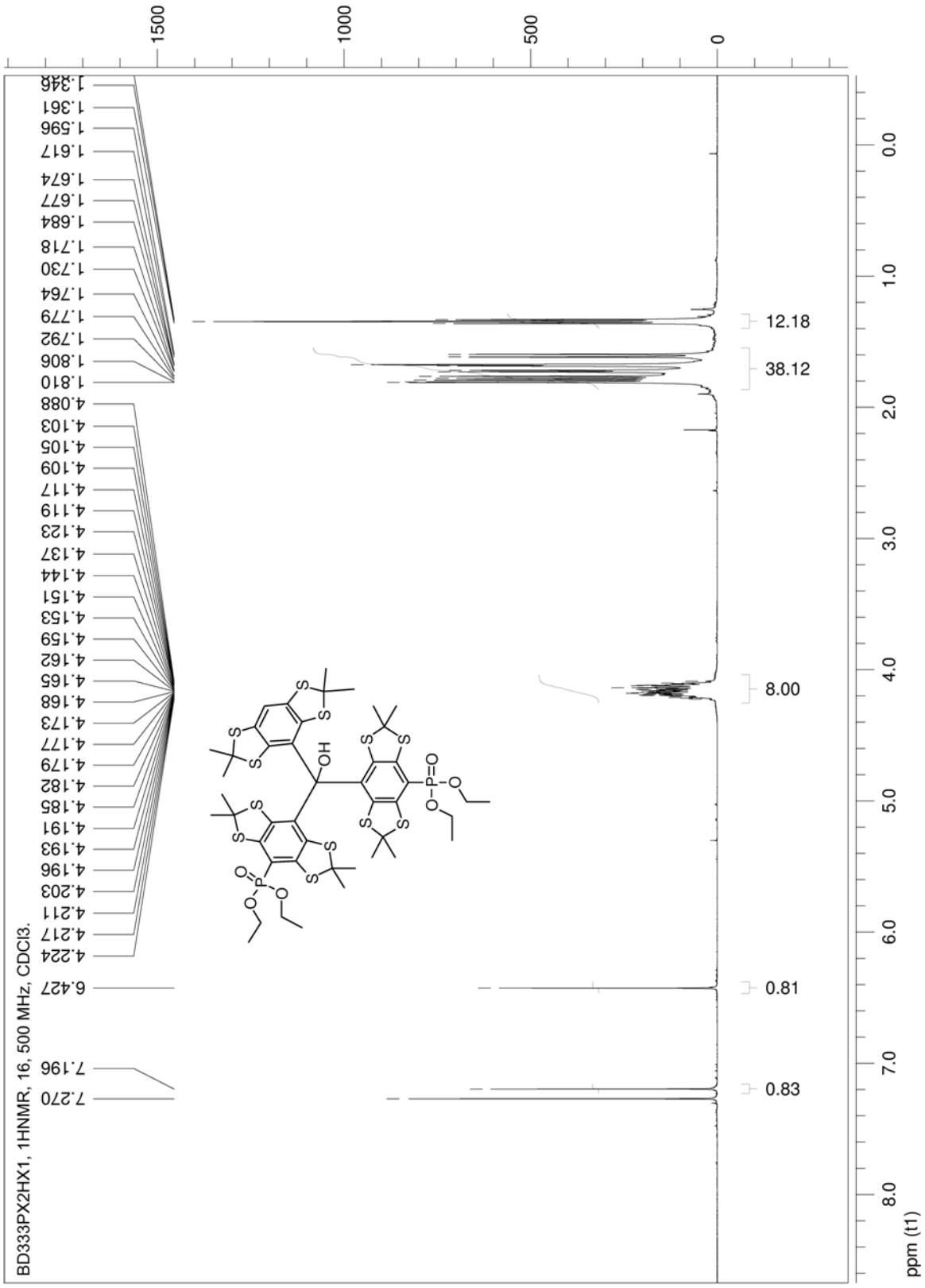


Figure S6: ^{1}H NMR 500 MHz spectrum of **2b**

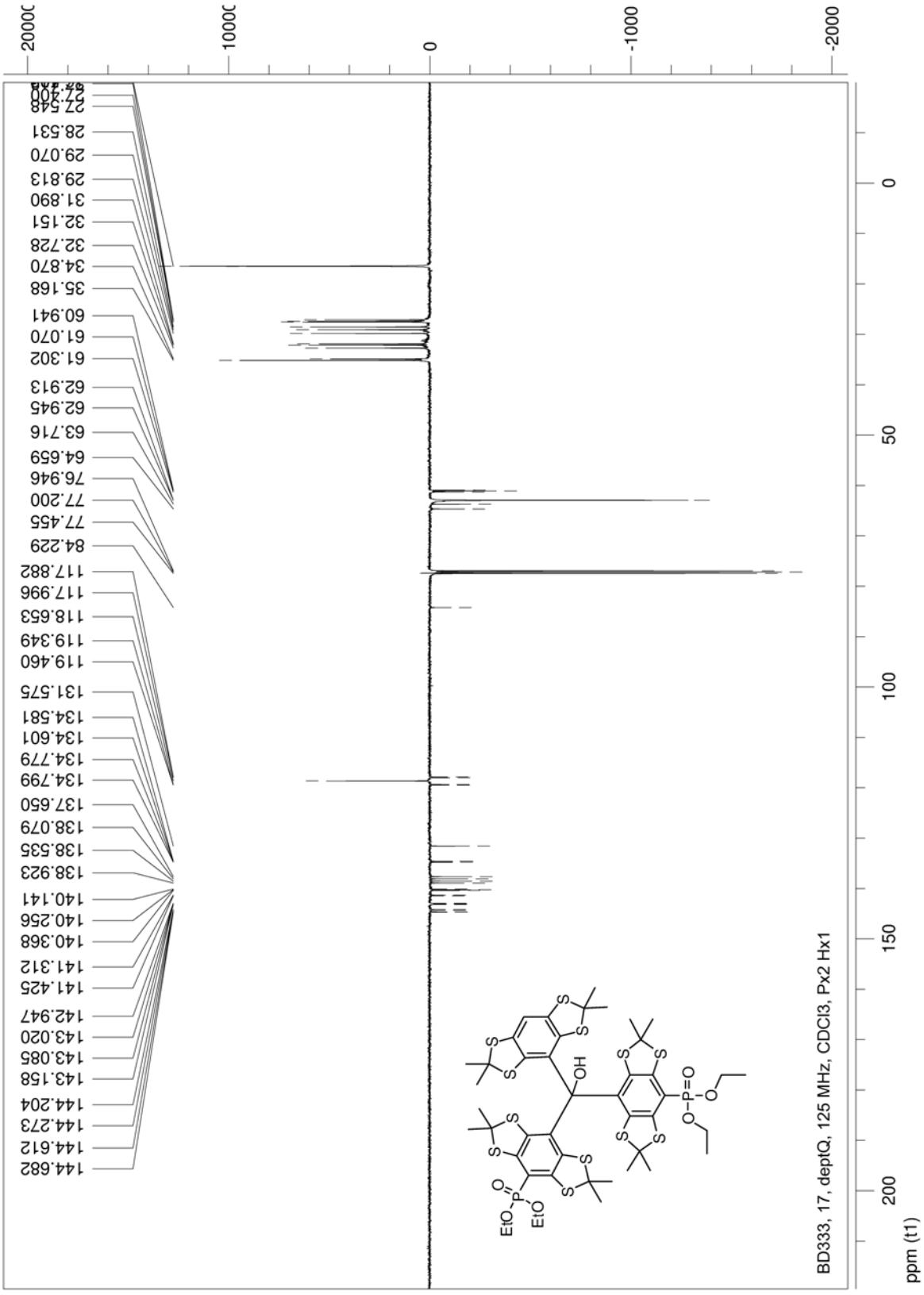


Figure S7: ^{13}C NMR 125 MHz spectrum of **2b**

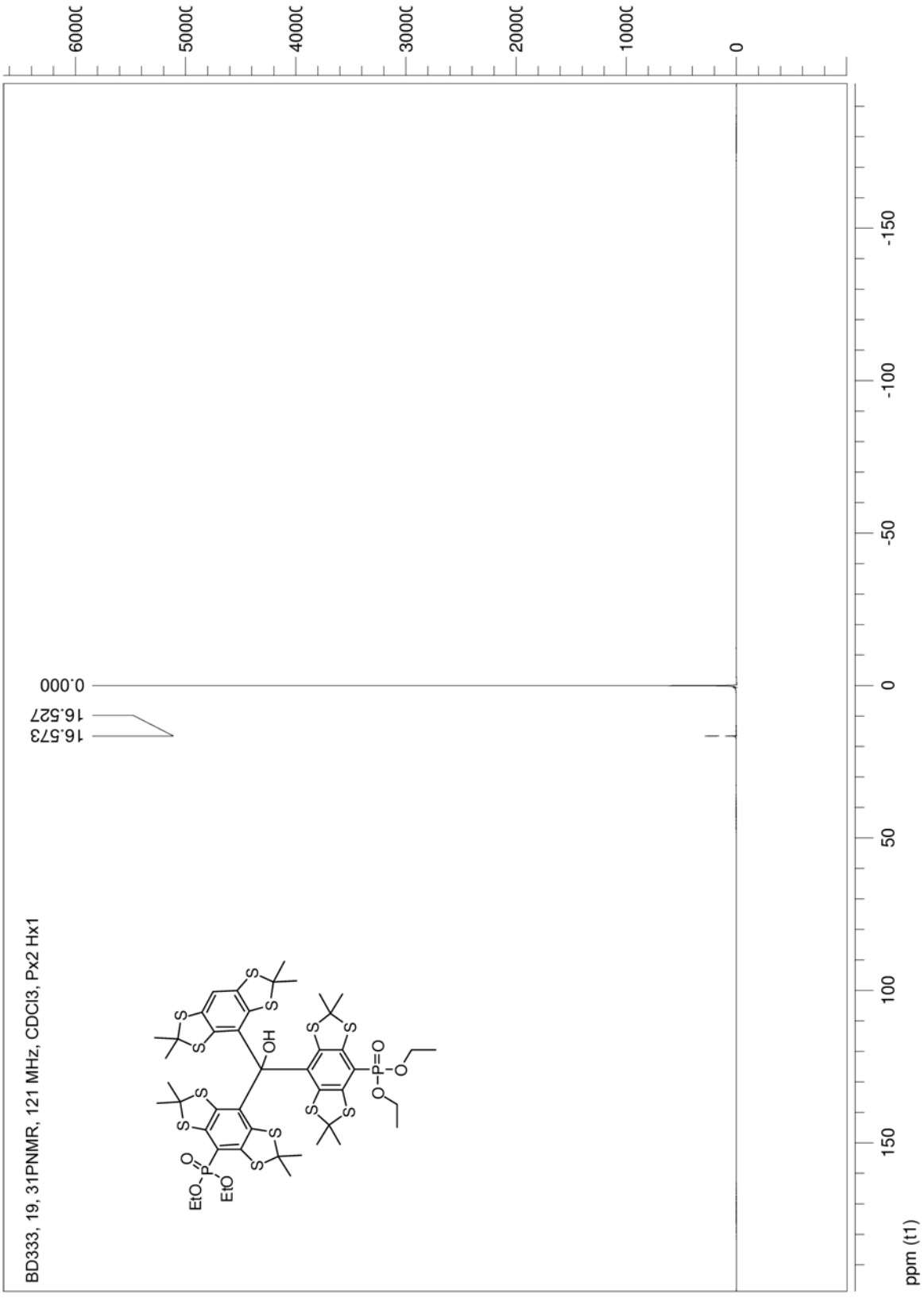


Figure S8: ^{31}P NMR 121 MHz spectrum of **2b**

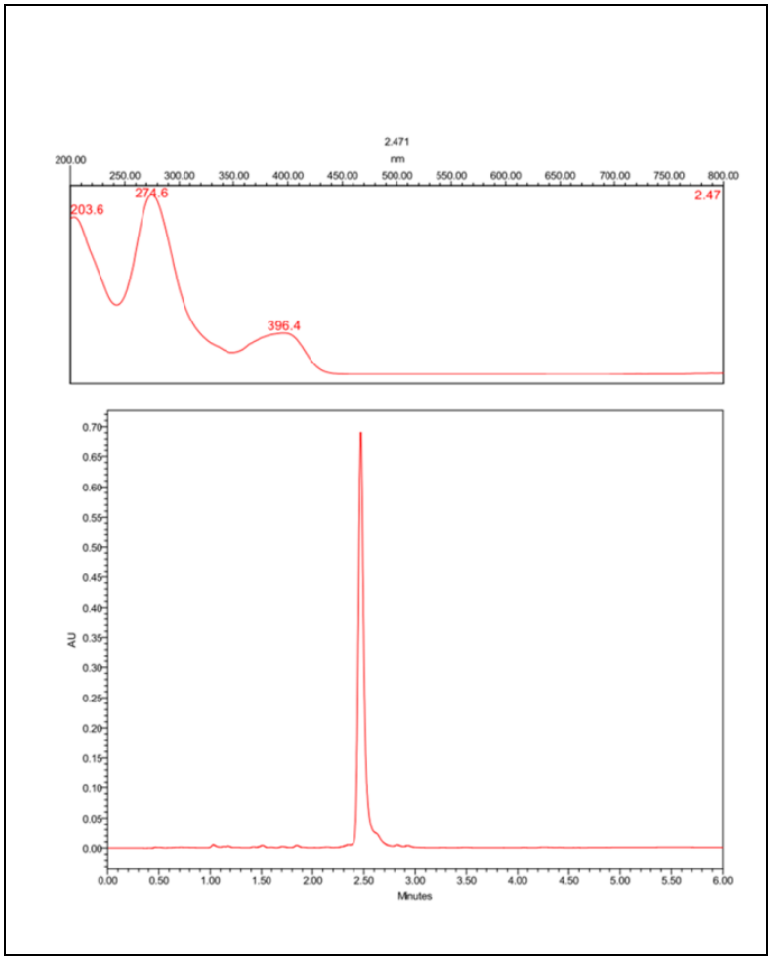


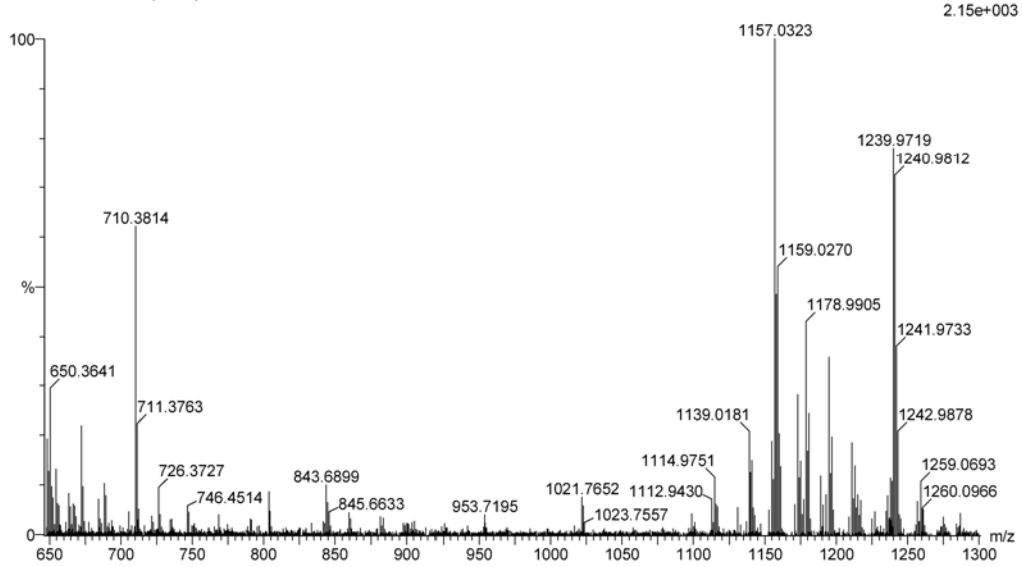
Figure S9: HPLC/UV of **2b**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
2 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 45-45 H: 4-250 O: 7-7 Na: 0-1 P: 2-2 S: 12-12

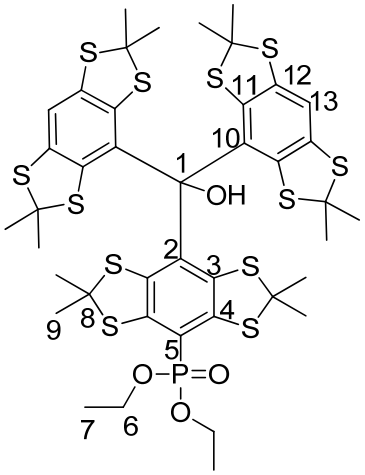
BD333PX2HX1a 10 (0.345) 1: TOF MS ES+ 2.15e+003



Minimum:				-1.5		
Maximum:		20.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
1157.0323	1157.0385	-6.2	-5.4	17.5	39.7	C45 H59 O7 P2 S12

Figure S10: HRMS of **2b**

8-diethoxyphosphoryl-tris(2,2,6,6-tetramethylbenzo[1,2-d;4,5-d']bis[1,3]dithiol-4-yl)methanol (**2c**)



Aspect: Orange solid.

Rf (SiO_2): 0.55 DCM/EtOAc (90:10) UV (254 nm).

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.35 (t, *J*=7.1 Hz, 6H, H-7), 1.61 (H-9), 1.67 (H-9'), 1.69 (H-9''), 1.69 (H-9'''), 1.72 (H-9$^{4'}$), 1.73 (H-9$^{5'}$), 1.77 (H-9$^{6'}$), 1.78 (H-9$^{7'}$), 1.79 (H-9$^{8'}$), 1.80 (H-9$^{9'}$), 1.83 (H-9$^{10'}$), 1.84 (H-9$^{11'}$), 4.05-4.25 (m, 4H, H-6), 6.33 (s, OH), 7.19 (s, 1H, H-13), 7.19 (s, 1H, H-13').

^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 16.5 (m, H-7), 27.3 (C-9), 27.5 (C-9'), 27.9 (C-9''), 28.6 (C-9'''), 29.3 (C-9$^{4'}$), 29.8 (C-9$^{5'}$), 31.8 (C-9$^{6'}$), 32.5 (C-9$^{7'}$), 32.6 (C-9$^{8'}$), 34.9 (C-9$^{9'}$), 34.9 (C-9$^{10'}$), 35.2 (C-9$^{11'}$), 61.0 (C-8), 61.0 (C-8'), 62.9 (m, C-6), 63.6 (C-8''), 63.6 (C-8'''), 64.2 (C-8$^{4'}$), 64.6 (C-8$^{5'}$), 84.0 (C-1), 118.4 (d, *J*=184.1 Hz, C-5), 118.5 (C-13), 118.6 (C-13'), 131.7 (C-ArH), 131.9 (C-ArH), 134.9 (d, *J*=2.4 Hz, C-2), 137.5 (C-ArH), 137.5 (C-ArH), 138.1 (C-ArH), 138.2 (C-ArH), 138.4 (C-ArH), 138.5 (C-ArH), 138.6 (C-ArH), 139.7 (C-ArH), 140.4 (d, *J*=14.3 Hz, C-3), 141.1 (d, *J*=14.2 Hz, C-3'), 143.0 (d, *J*=9.1 Hz, C-4), 144.2 (d, *J*=8.9 Hz, C-4').

^{31}P NMR (121 MHz, $CDCl_3$) δ (ppm): 16.8.

ATR-IR (cm^{-1}): 2959, 2918, 1452, 1365, 1250, 1149, 1020.

HRMS (TOF MS ES+) *m/z*: $[M+H]^+$ calcd for $C_{41}H_{50}O_4P_1S_{12}$: 1021.0095, found: 1021.0020.

RP-HPLC: RT=3.8 min, column: XBridge C18 2.5 µm 4.6 x 50mm equipped with a guard column XBridge C18 2.5 µm 4.6 x 20mm. Column temperature: 40°C, UV detection at 254 nm.

Time	Flow	H_2O	ACN	Curve (Waters)
	1.5 mL/min	20%	80%	
4.00 min	1.5 mL/min	0%	100%	6 (linear)
6.00 min	1.5 mL/min	0%	100%	6
6.10 min	1.5 mL/min	20%	80%	6

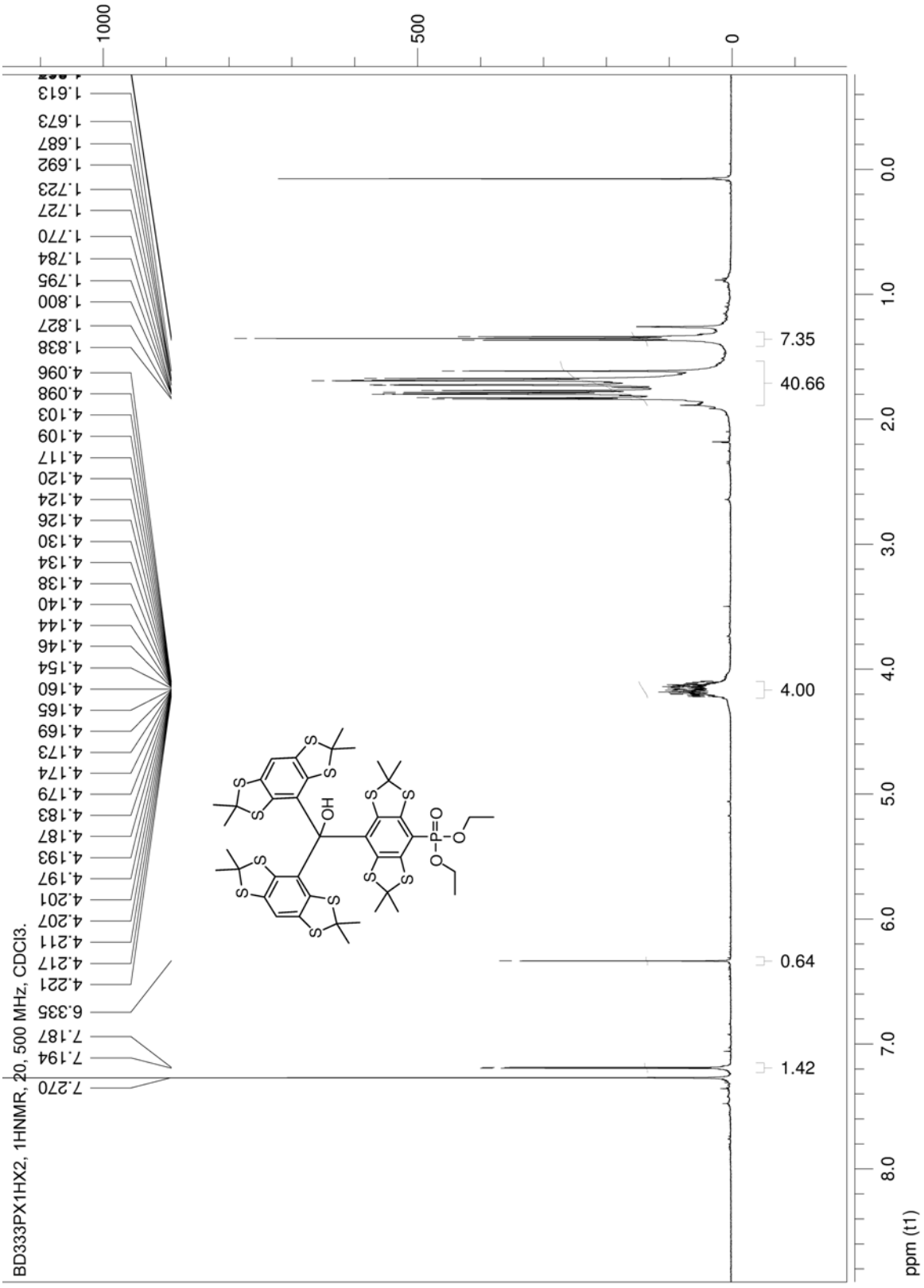


Figure S11: ^{1}H NMR 500 MHz spectrum of **2c**

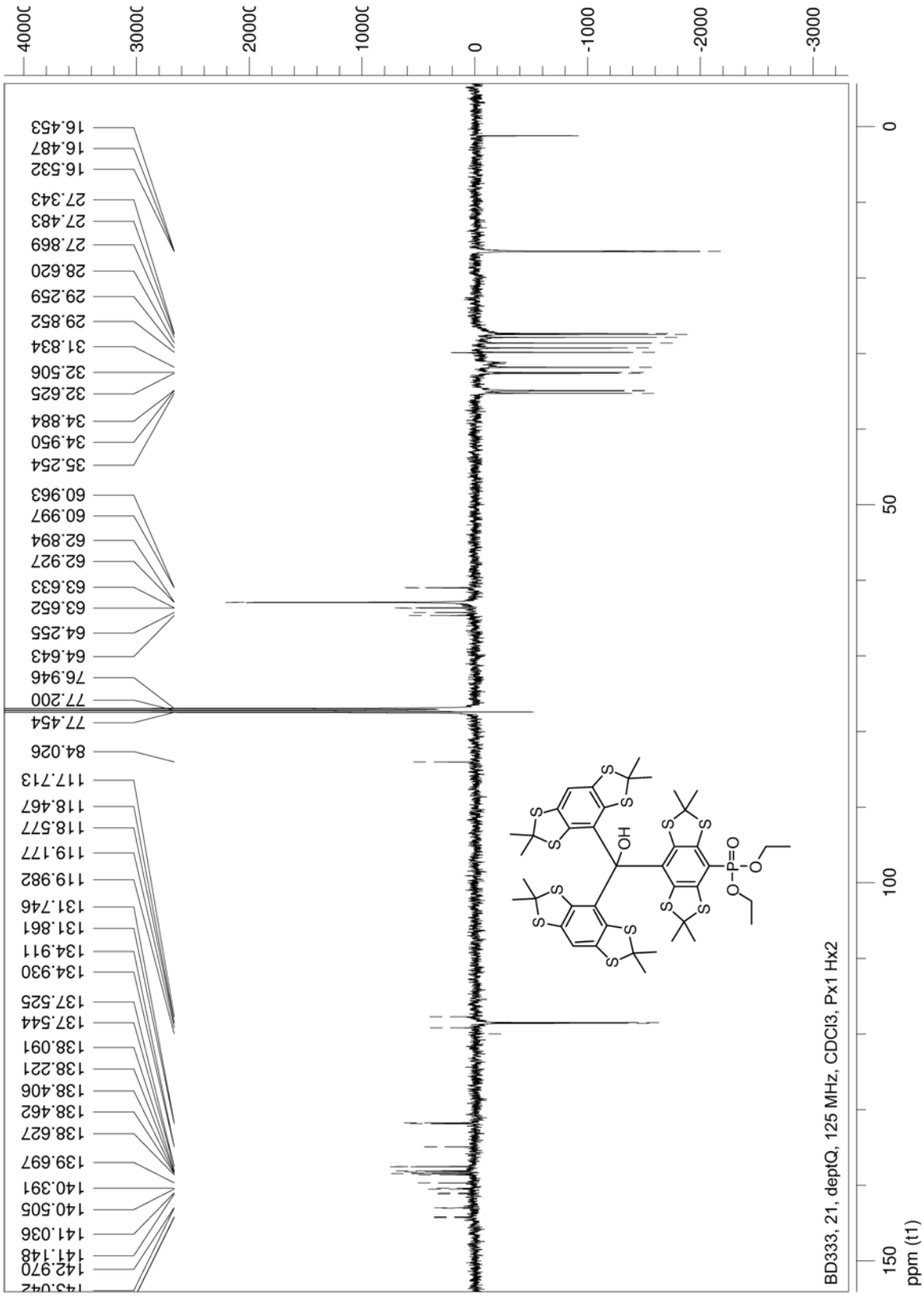


Figure S12: ^{13}C NMR 125 MHz spectrum of **2c**

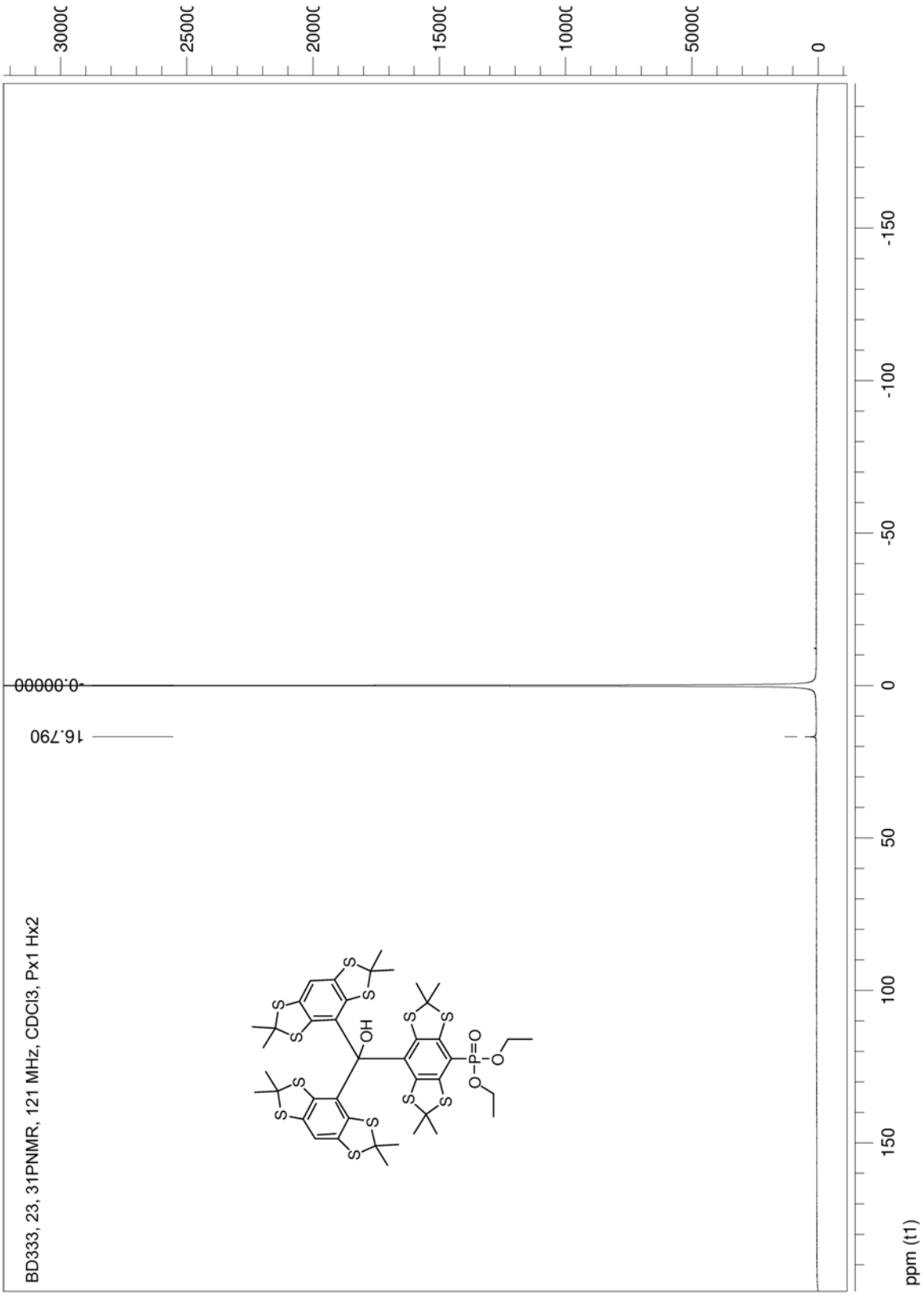


Figure S13: ^{31}P NMR 121 MHz spectrum of **2c**

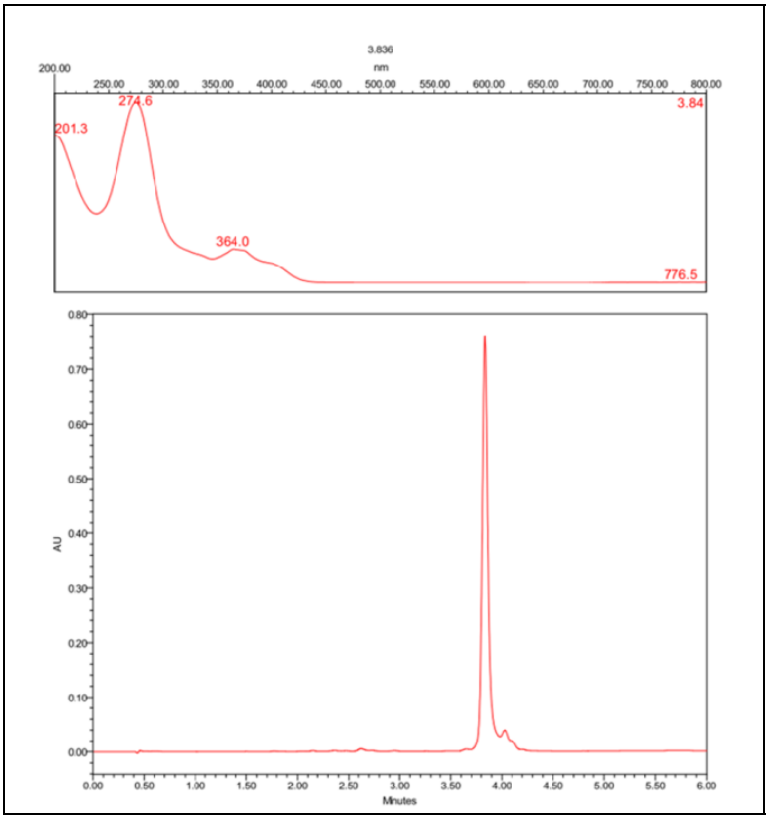


Figure S14: HPLC/UV of **2c**

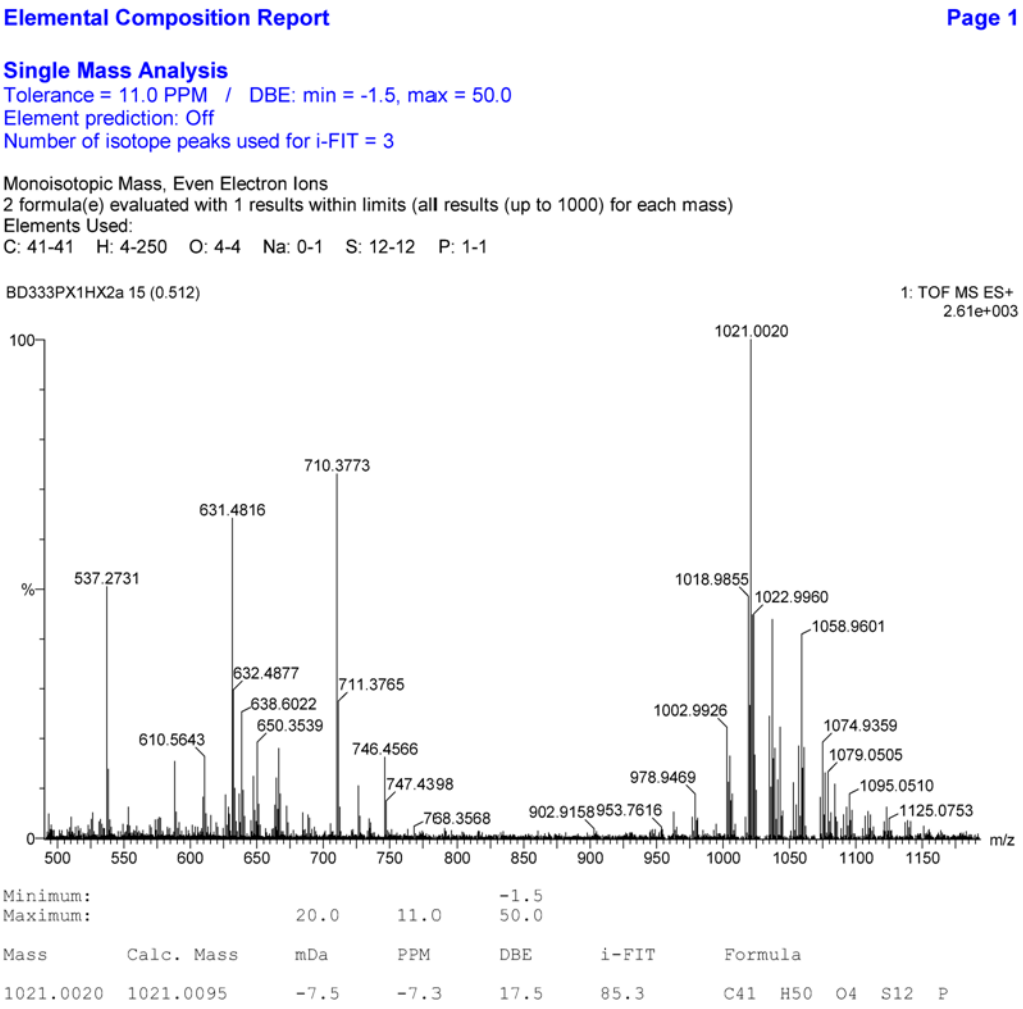
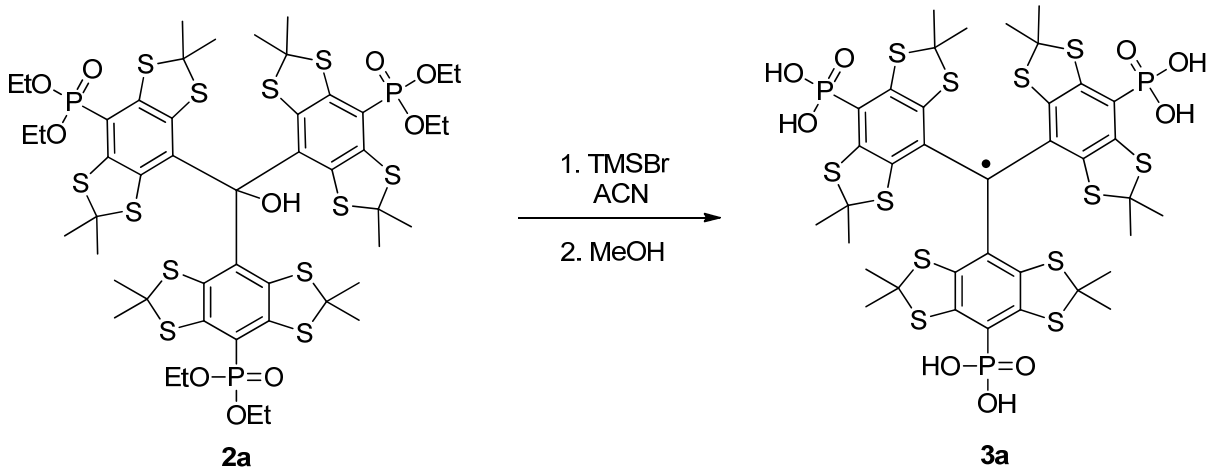


Figure S15: HRMS of **2c**

2.2 Synthesis of Tris(8-phosphono-2,2,6,6-tetramethylbenzo[1,2-d;4,5-d']bis[1,3]dithiol-4-yl)methyl (3a)



2a **3a**

To a solution of trityl alcohol **2a** (110 mg, 0.085 mmol, 1 eq.) in CH_3CN (12 mL) was added TMSBr (169 µL, 1.275 mmol, 15 eq.) The mixture was refluxed for 22h, the orange solution gradually turned green. The solvent was removed under reduced pressure and the green residue was then dissolved and stirred at room temperature for 2 hours in MeOH (15 mL). After evaporation of MeOH, the green residue was purified by semi-preparative HPLC to afford the title compound (59 mg, 60%) as an ammonium salt.

The conditions of purification were as follows:

Column: XBridge Prep C18 5 µM 10 x 100 mm at ambient temperature equipped with a guard column XBridge Prep C18 5 µM 10 x 20 mm, helium sparge 30 mL/min, UV detection at 254 nm. For high purity, the product was collected from the half-height of the upward slope to the half-height of the downward slope. Gradient elution:

Time	Flow	H_2O	ACN	NH_4OAc 500 mM	Curve (Waters)
0.00 min	5 mL/min	85%	5%	10%	
6.00 min	5 mL/min	75%	15%	10%	6 (linear)
8.00 min	5 mL/min	75%	15%	10%	6
9.00 min	5 mL/min	10%	90%	0%	6
11.00 min	5 mL/min	10%	90%	0%	6
11.10 min	5 mL/min	85%	5%	10%	6

N.B: small amounts of deprotected phosphonic acids with residual trityl alcohol might be present in the crude. In that case, trityl alcohol can be converted into a radical by dissolving the crude mixture in TFA (15 mL) for 1h before purification.

Aspect: Green solid.

HRMS (TOF MS ES+) *m/z*: $[M+H]^+$ calcd for $C_{37}H_{43}O_9P_3S_{12}$: 1107.8769, found: 1107.9026.

RP-HPLC: RT=1.6 min, column: XBridge C18 2.5 µm 4.6 x 50mm. Column temperature: 40°C, UV detection at 254 nm.

Time	Flow	H_2O	ACN	NH_4OAc 50 mM	Curve (Waters)
	1.5 mL/min	85%	5%	10%	
3.00 min	1.5 mL/min	75%	15%	10%	6 (linear)
4.00 min	1.5 mL/min	0%	90%	10%	6
6.00 min	1.5 mL/min	0%	90%	10%	6
6.10 min	1.5 mL/min	85%	5%	10%	6

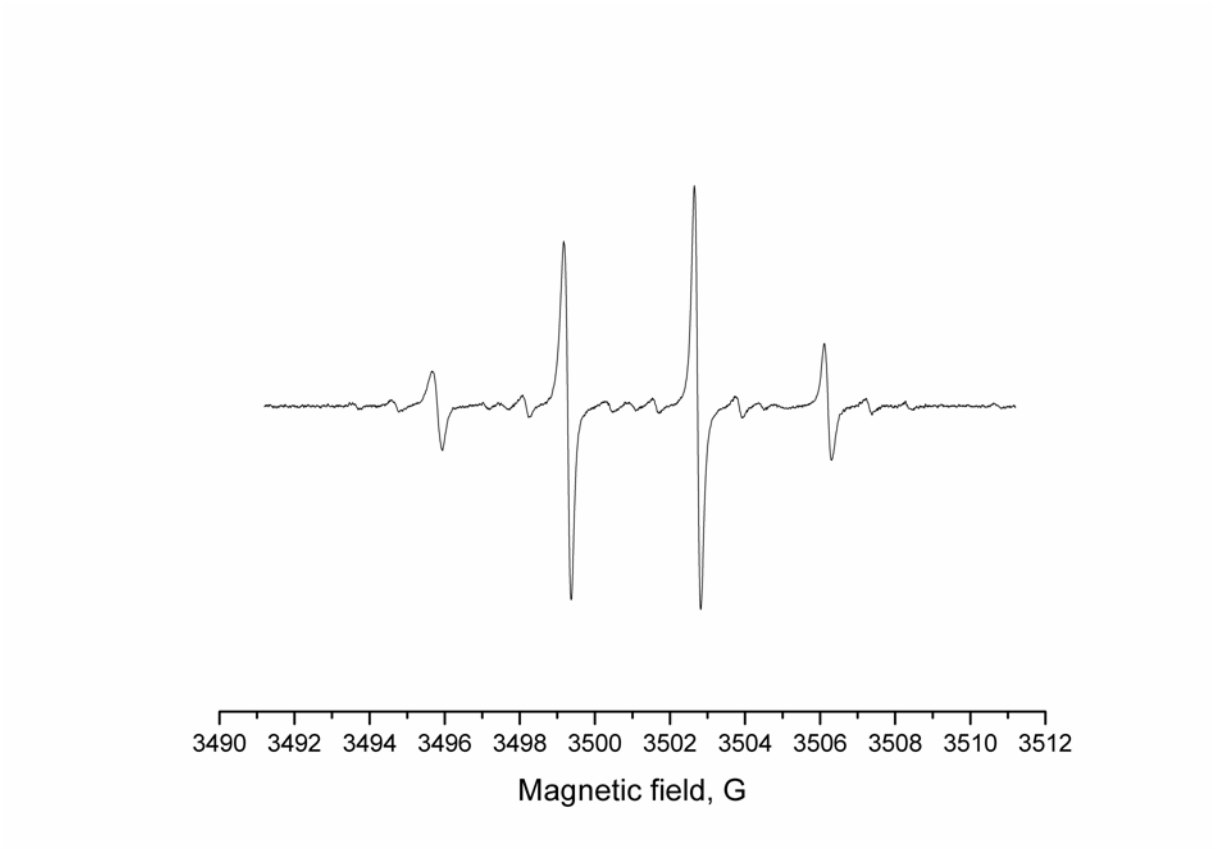


Figure S16: X-Band EPR spectrum of **3a** 100 µM in PBS 10 mM, pH=7.51

Acquisition settings were: power, 0.5 mW; sweep width, 20 G; modulation frequency, 10 kHz; modulation amplitude, 0.02 G; time constant, 20.48 ms; conversion time, 20.48 ms; sweep time, 41.94 sec; number of points, 2048; number of scans, 3; room temperature; normal air.

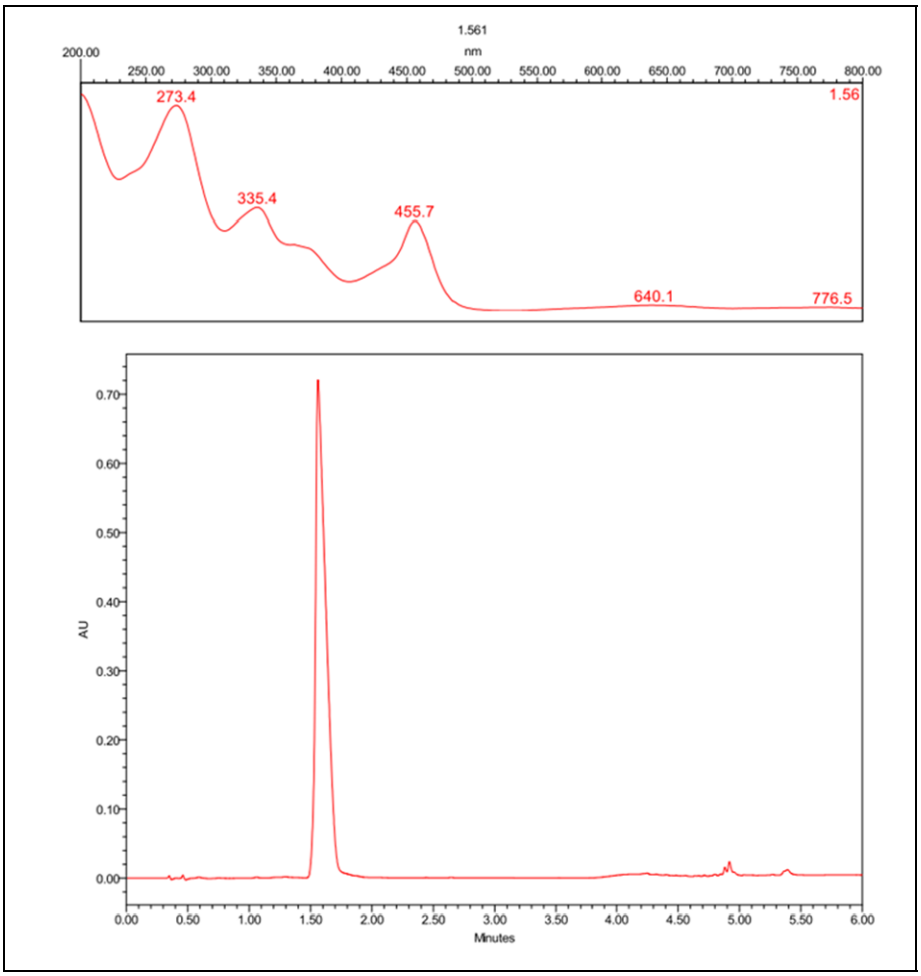


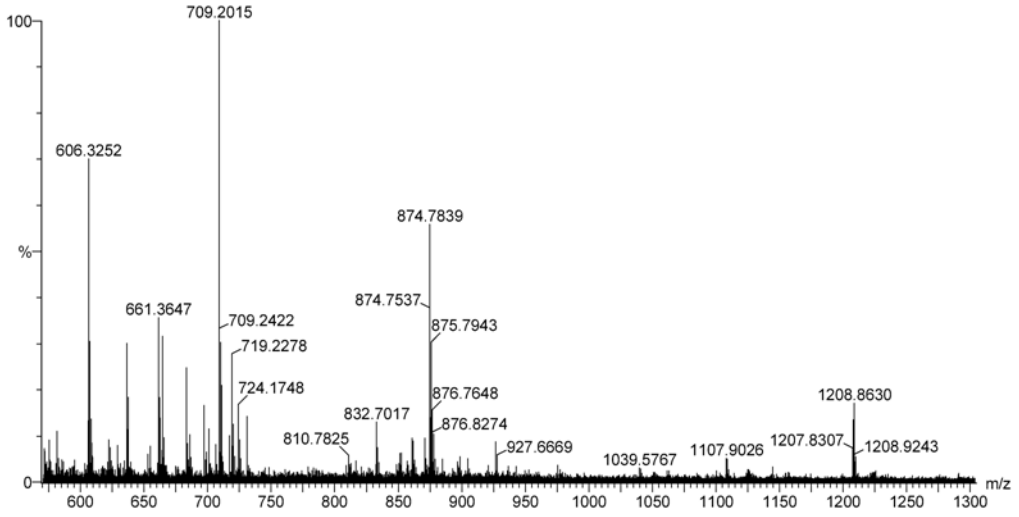
Figure S17: HPLC/UV of **3a**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 100.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Odd and Even Electron Ions
8 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 37-37 H: 4-200 O: 9-9 Na: 0-1 P: 3-3 S: 12-12 I: 0-3

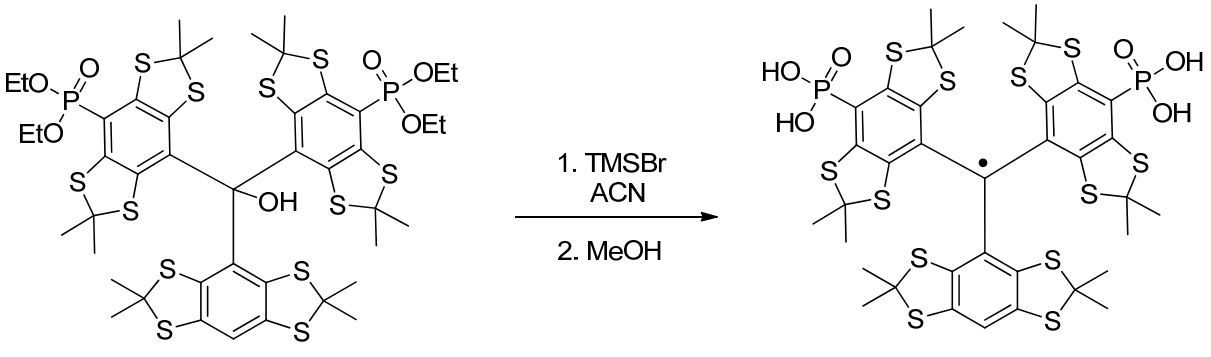
LCT Premier
17-Oct-2011
BD385B 16 (0.359)
1: TOF MS ES+
4.80e+003



Minimum:				-1.5		
Maximum:		20.0	100.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
1107.9026	1107.8769	25.7	23.2	18.0	22.1	C37 H43 O9 P3 S12

Figure S18: HRMS of **3a**

2.3 Synthesis of Bis(8-phosphono)tris(2,2,6,6-tetramethylbenzo[1,2-d;4,5-d']bis[1,3]dithiol-4-yl)methyl (3b)



2b **3b**

To a solution of trityl alcohol **2b** (35 mg, 0.030 mmol, 1 eq.) in CH_3CN (3 mL) was added TMSBr (60 µL, 0.453 mmol, 15 eq.) The mixture was refluxed for 22h, the orange solution gradually turned green. The solvent was removed under reduced pressure and the green residue was then dissolved and stirred at room temperature for 2 hours in MeOH (5 mL). After evaporation of MeOH, the green residue was purified by semi-preparative HPLC to afford the title compound (3.4 mg, 11%) as an ammonium salt.

The conditions of purification were as follows:

Column: XBridge Prep C18 5 µM 10 x 100 mm at ambient temperature equipped with a guard column XBridge Prep C18 5 µM 10 x 20 mm, helium sparge 30 mL/min, UV detection at 273 nm. For high purity, the product was collected from the half-height of the upward slope to the half-height of the downward slope. Isocratic elution: 33%ACN/57%H_2O/10%NH_4OAc 500 mM, flow: 5 mL/min.

N.B: small amounts of deprotected phosphonic acids with residual trityl alcohol might be present in the crude. In that case, trityl alcohol can be converted into a radical by dissolving the crude mixture in TFA (5 mL) for 1h before purification.

Aspect: Green solid.

HRMS (TOF MS ES+) *m/z*: $[M+H]^+$ calcd for $C_{37}H_{42}O_6P_3S_{12}$: 1027.9105, found: 1027.9109.

RP-HPLC: RT=5.1 min, column: XBridge C18 2.5 µm 4.6 x 50mm equipped with a guard column XBridge C18 2.5 µm 4.6 x 20mm. Column temperature: 40°C, UV detection at 254 nm.

Time	Flow	H_2O	ACN	NH_4OAc 50 mM	Curve (Waters)
	1.5 mL/min	85%	5%	10%	
10.00 min	1.5 mL/min	0%	90%	10%	6 (linear)
12.00 min	1.5 mL/min	0%	90%	10%	6
12.10 min	1.5 mL/min	85%	5%	10%	6

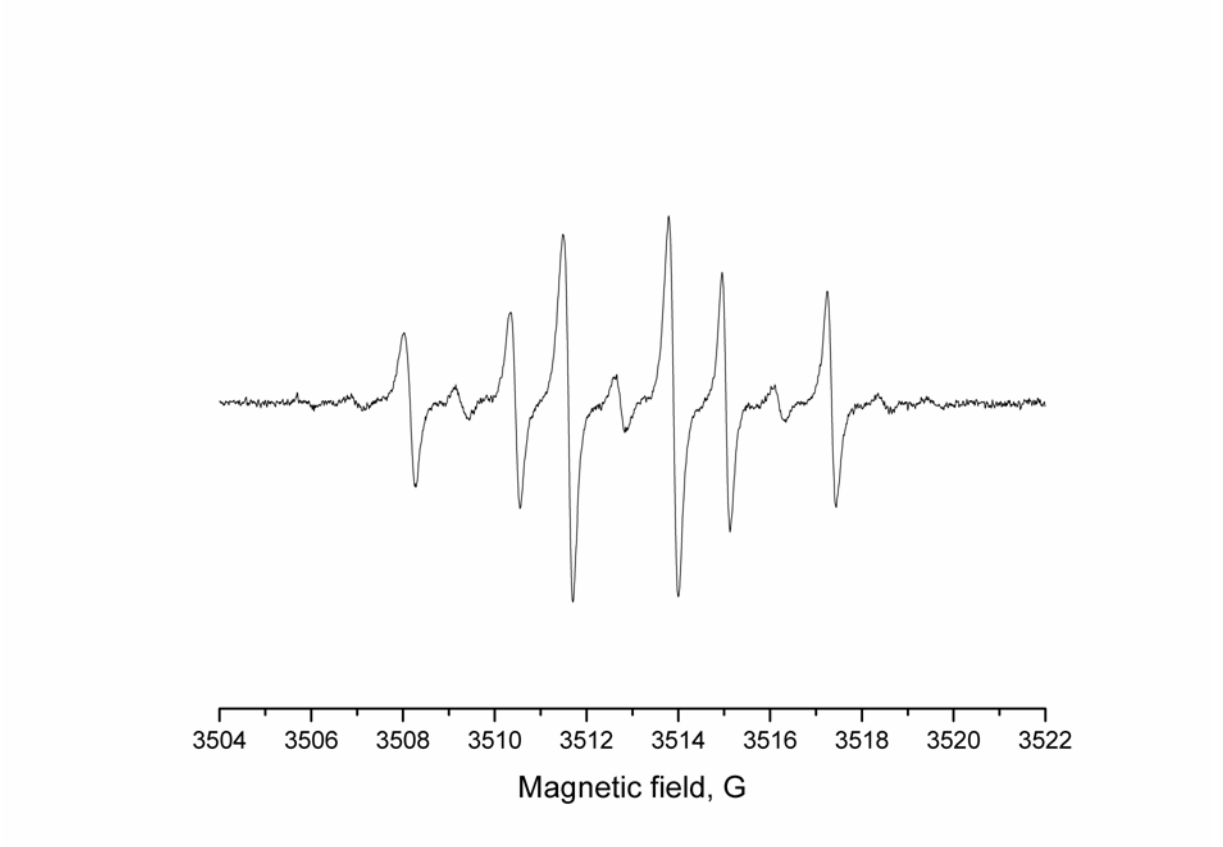


Figure S19: X-Band EPR spectrum of **3b** 100 µM in PBS 10 mM, pH=7.46

Acquisition settings were: power, 0.5 mW; sweep width, 20 G; modulation frequency, 10 kHz; modulation amplitude, 0.02 G; time constant, 20.48 ms; conversion time, 20.48 ms; sweep time, 41.94 sec; number of points, 2048; number of scans, 10; room temperature; normal air.

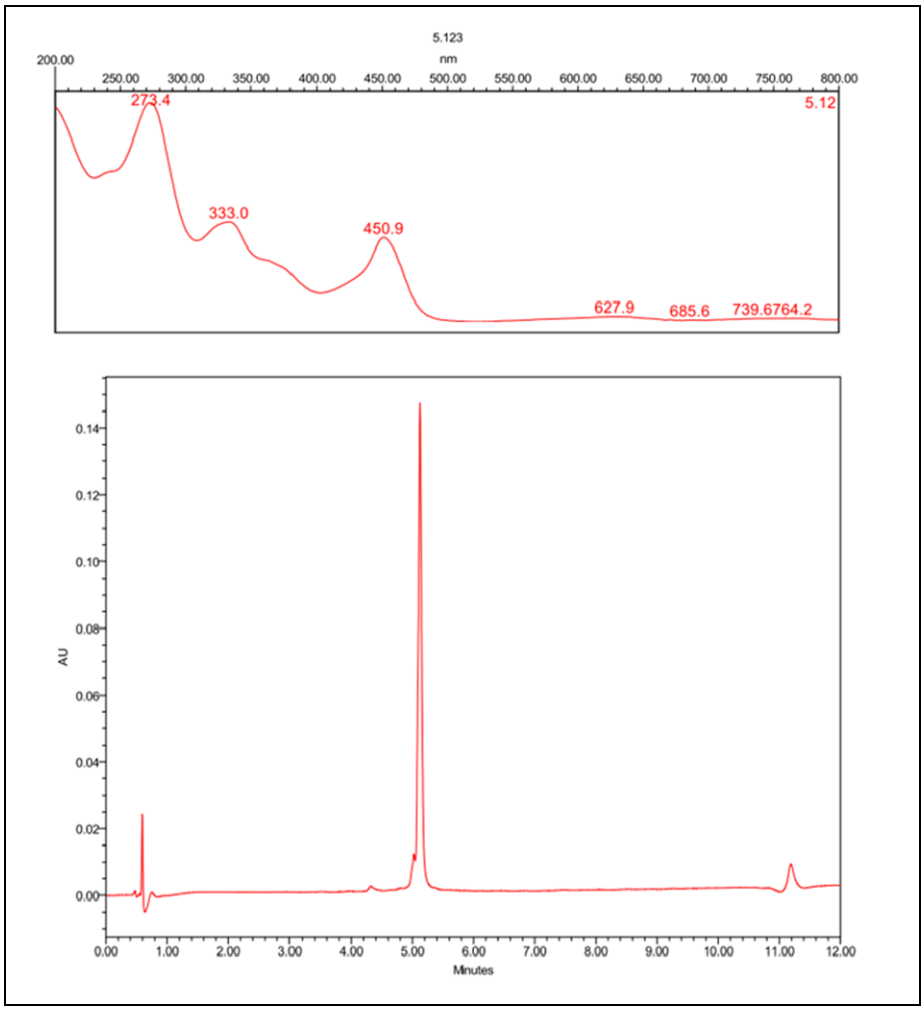


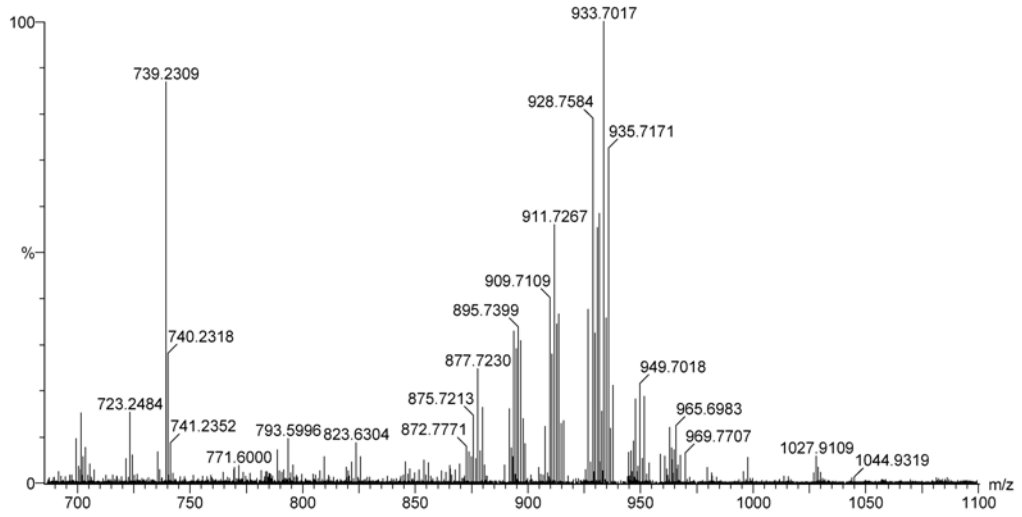
Figure S20: HPLC/UV of **3b**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Odd and Even Electron Ions
2 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 37-37 H: 4-200 O: 6-6 Na: 0-1 P: 2-2 S: 12-12

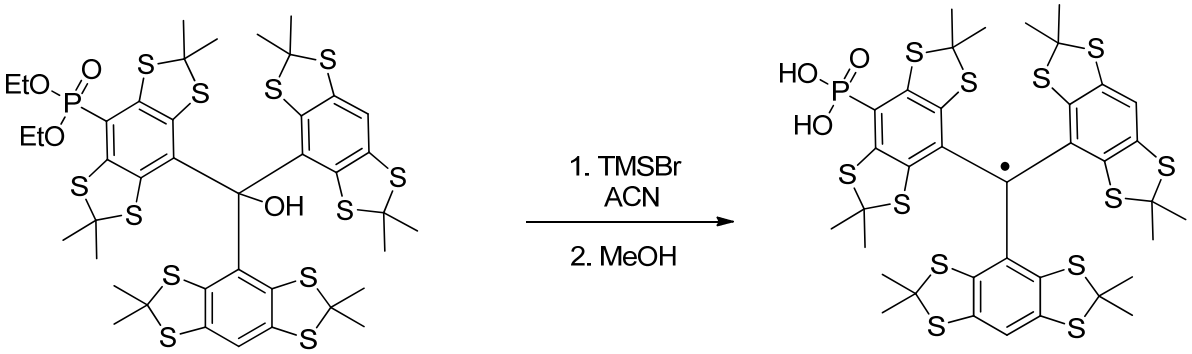
LCT Premier
17-Jun-2011
BD377FR1A 9 (0.316) Cm (9:12)
1: TOF MS ES+
6.18e+003



Minimum:				-1.5		
Maximum:		20.0	20.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
1027.9109	1027.9105	0.4	0.4	18.0	1.1	C37 H42 O6 P2 S12

Figure S21: HRMS of **3b**

2.4 Synthesis of 8-phosphono-tris(2,2,6,6-tetramethylbenzo[1,2-d;4,5-d']bis[1,3]dithiol-4-yl)methyl (3c)



2c **3c**

To a solution of trityl alcohol **2c** (9 mg, 0.009 mmol, 1 eq.) in CH_3CN (1 mL) was added TMSBr (17.5 µL, 0.132 mmol, 15 eq.) The mixture was refluxed for 22h, the orange solution gradually turned green. The solvent was removed under reduced pressure and the green residue was then dissolved and stirred at room temperature for 2 hours in MeOH (5 mL). After evaporation of MeOH, the green residue was purified by semi-preparative HPLC to afford the title compound (1.1 mg, 12%) as an ammonium salt.

The conditions of purification were as follows:

Column: XBridge Prep C18 5 µM 10 x 100 mm at ambient temperature equipped with a guard column XBridge Prep C18 5 µM 10 x 20 mm, helium sparge 30 mL/min, UV detection at 273 nm. For high purity, the product was collected from the half-height of the upward slope to the half-height of the downward slope. Isocratic elution: 65%ACN/25%H_2O/10%NH_4OAc 500 mM, flow: 5 mL/min.

N.B: small amounts of deprotected phosphonic acids with residual trityl alcohol might be present in the crude. In that case, trityl alcohol can be converted into a radical by dissolving the crude mixture in TFA (5 mL) for 1h before purification.

Aspect: Green solid.

HRMS (TOF MS ES+) *m/z*: $[M+H]^+$ calcd for $C_{37}H_{41}O_3PS_{12}$: 947.9442, found: 947.9473.

RP-HPLC: RT=9.1 min, column: XBridge C18 2.5 µm 4.6 x 50mm equipped with a guard column XBridge C18 2.5 µm 4.6 x 20mm. Column temperature: 40°C, UV detection at 254 nm.

Time	Flow	H_2O	ACN	NH_4OAc 50 mM	Curve (Waters)
	1.5 mL/min	85%	5%	10%	
10.00 min	1.5 mL/min	0%	90%	10%	6 (linear)
12.00 min	1.5 mL/min	0%	90%	10%	6
12.10 min	1.5 mL/min	85%	5%	10%	6

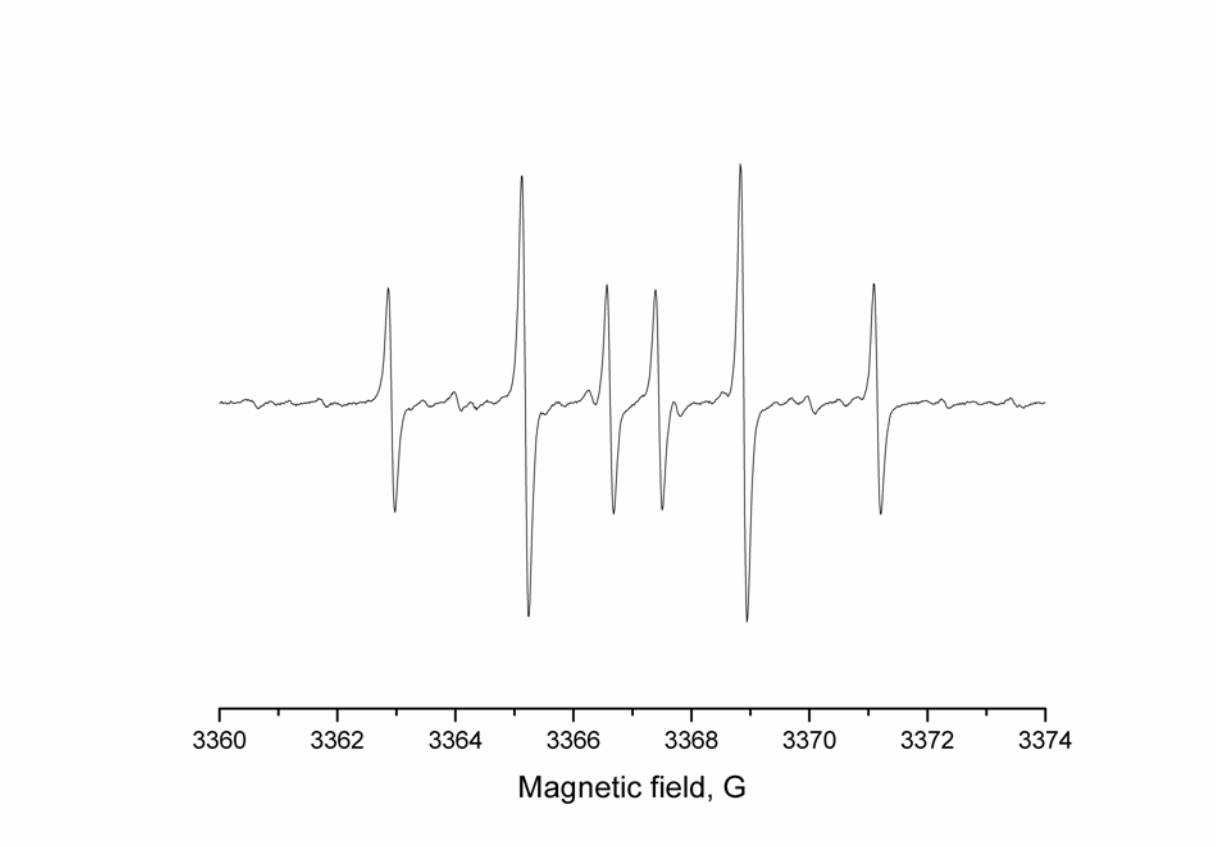


Figure S22: X-Band EPR spectrum of **3c** 100 µM in degassed MeOH.

Acquisition settings were: power, 0.3 mW; sweep width, 26 G; modulation frequency, 10 kHz; modulation amplitude, 0.03 G; time constant, 20.48 ms; conversion time, 40.96 ms; sweep time, 83.89 sec; number of points, 2048; number of scans, 3; room temperature.

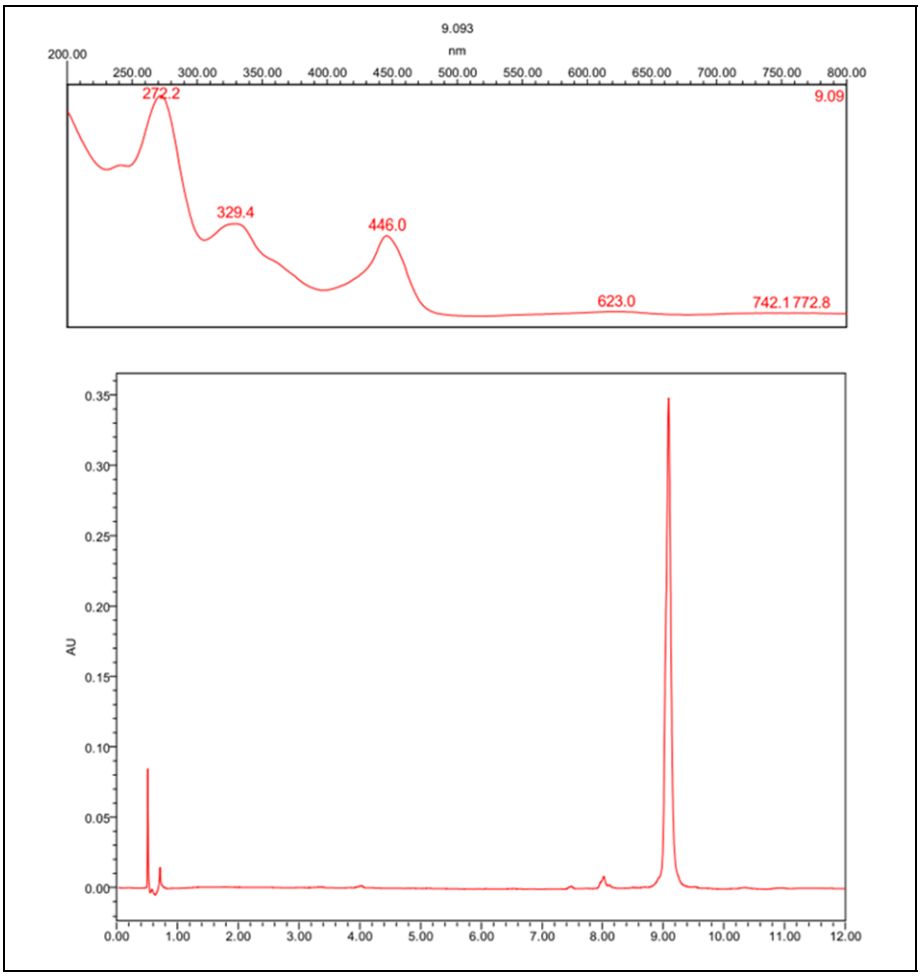


Figure S23: HPLC/UV of **3c**

Elemental Composition Report **Page 1**

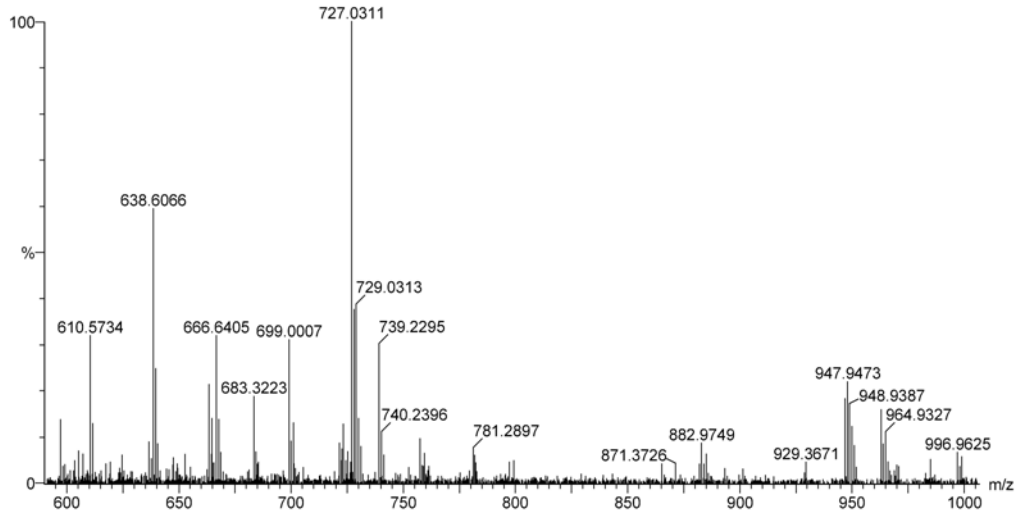
Single Mass Analysis
Tolerance = 100.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Odd and Even Electron Ions
7 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 37-37 H: 4-200 O: 3-6 Na: 0-1 P: 1-1 S: 12-12

LCT Premier

16-Jun-2011
BD378FR1_2 9 (0.317) Cm (9-4:5)

1: TOF MS ES+
1.27e+003



Minimum:				-1.5		
Maximum:		20.0	100.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
947.9473	947.9442	3.1	3.3	18.0	9.3	C37 H41 O3 P S12

Figure S24: HRMS of **3c**

3. Evaluation of 3a as EPR pH probe

3.1 Saturation

Saturation plot of **3a** 100 µM in PBS (10 mM PB, 138 mM NaCl, 2.7 mM KCl) pH=7.46

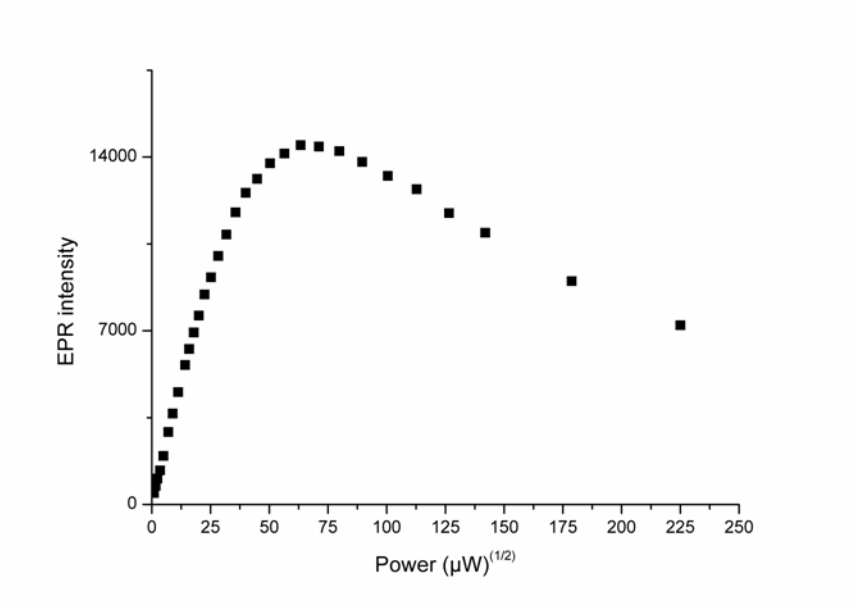


Figure S25: Saturation of **3a** under normal air condition

Acquisition settings were: power, from 1 µW to 50.6 mW; sweep width, 20 G; modulation frequency, 10 kHz; modulation amplitude, 0.02 G; time constant, 20.48 ms; conversion time, 20.48 ms; sweep time, 41.94 sec; number of points, 2048; number of scan, 1; room temperature; normal air.

3.2 Titration of 3a in PBS 10 mM

The X-band pH dependence of the apparent hyperfine splitting a_P and $3a_P$ were determined by conventional titration procedure of compound **3a** 100 µM in aerated PBS (10 mM PB, 138 mM NaCl, 2.7 mM KCl) at room temperature using NaOH and HCl.

Apparent hyperfine splitting constants (a_P) were measured as the distance between the two high-field components after integration of the signal as depicted in figure S26.

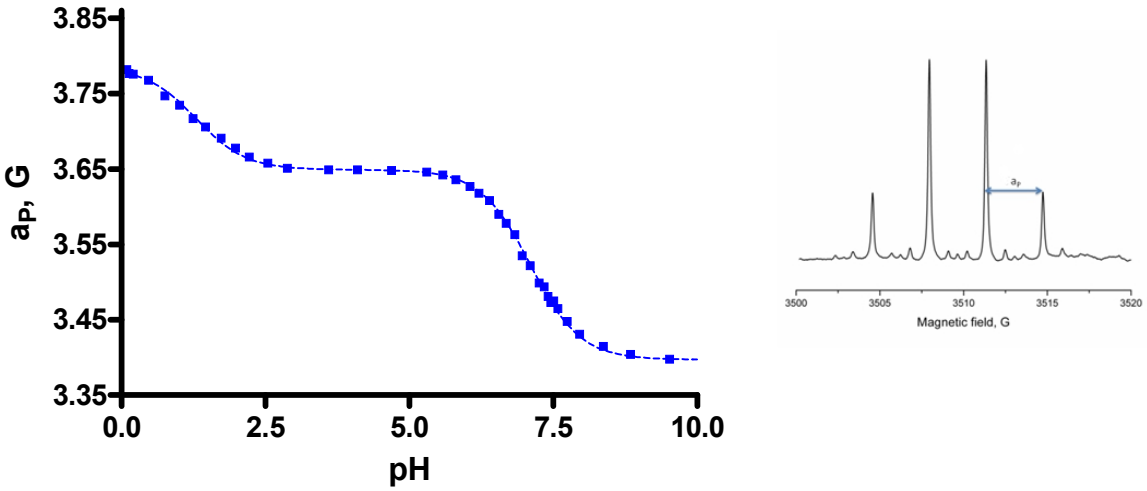


Figure S26: The X-band pH dependence of the apparent a_P

Acquisition settings were: power, 0.5 mW; sweep width, 20 G; modulation frequency, 10 kHz; modulation amplitude, 0.02 G; time constant, 20.48 ms; conversion time, 20.48 ms; sweep time, 41.94 sec; number of points, 2048; number of scans, 2.

Experimental data were fitted (dotted line) according equation (1). This equation is the standard titration dependence for a probe with two pKa values in the case of fast frequency proton exchanges in the EPR time scale. However, this equation is used as a good approximation for the case of moderate and slow frequency exchanges when the exchangeable lines are partially overlapped. The parameters of fitting were a_{P1} =3.785 G, a_{P2} =3.649 G, a_{P3} =3.397 G, pKa_1 = 1.293 and pKa_2 = 7.104.

$$a_P(pH) = \frac{a_{P_1} + a_{p_2} \times 10^{(-pKa_1 + pH)} + a_{P_3} \times 10^{(-pKa_1 - pKa_2 + 2 \times pH)}}{1 + 10^{(-pKa_1 + pH)} + 10^{(-pKa_1 - pKa_2 + 2 \times pH)}} \quad (1)$$

Apparent hyperfine splittings ($3a_P$) were measured as the distance between the low- and high-field components after integration of the signal as depicted in figure S27. Two modulation amplitudes were used, 0.02 G and 0.6 G.

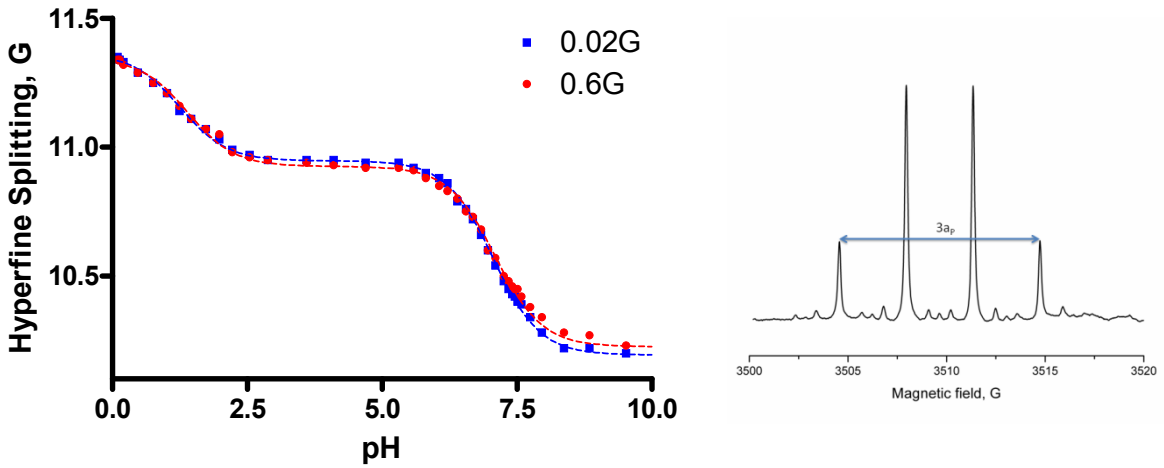
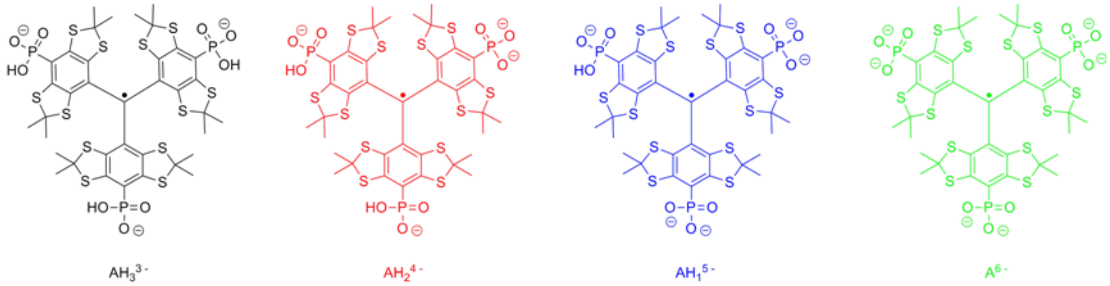


Figure S27: The X-band pH dependence of the apparent $3a_P$ at 0.02 G and 0.6 G modulation amplitude

Acquisition settings were: power, 0.5 mW; sweep width, 20 G; modulation frequency, 10 kHz; modulation amplitude, 0.02 G or 0.6 G; time constant, 20.48 ms; conversion time, 20.48 ms; sweep time, 41.94 sec; number of points, 2048; number of scans, 2.

3.3 X-band spectra of 3a 100 µM in PB 2 mM

In phosphate buffer 2 mM for pH close to the second pKa, the four ionic species (AH_3^{3-}, AH_2^{4-}, AH^{5-}, A^{6-}) are partially resolved on the spectrum under normal air conditions as a consequence of a slow proton frequency exchanges on the EPR time scale.



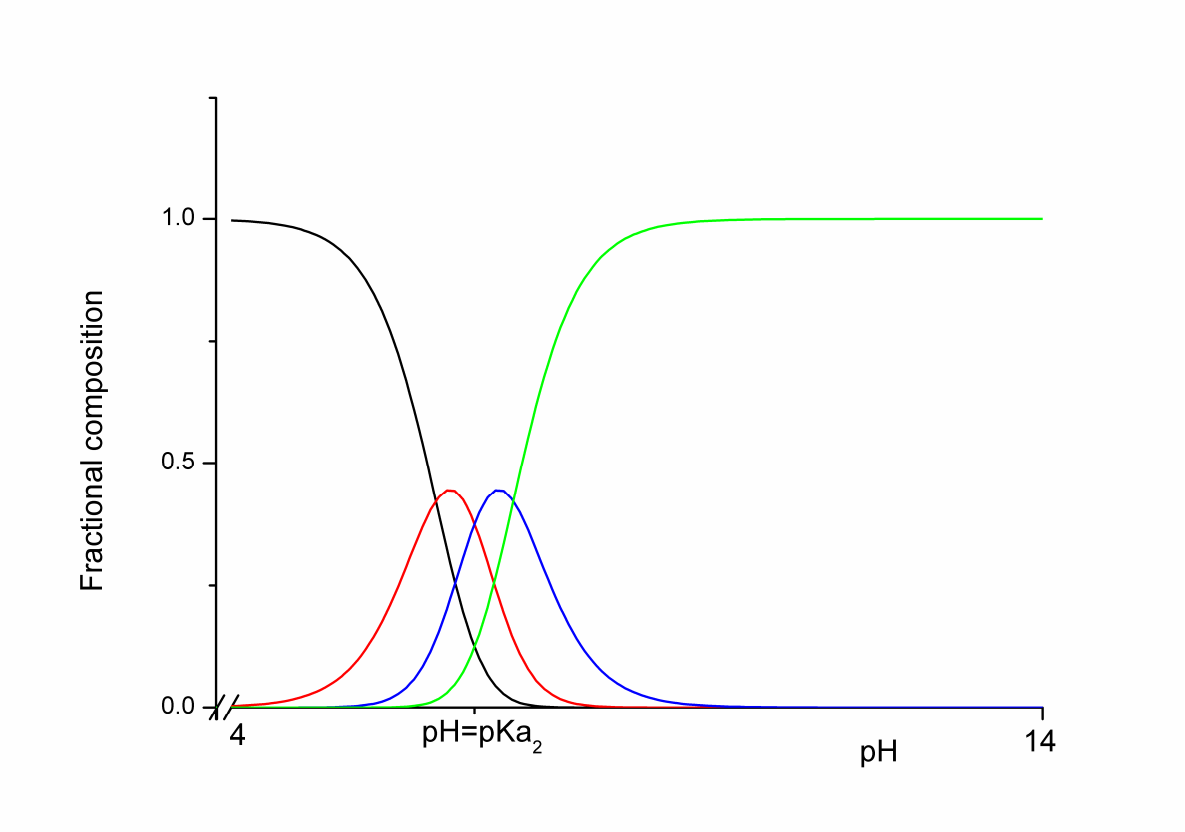
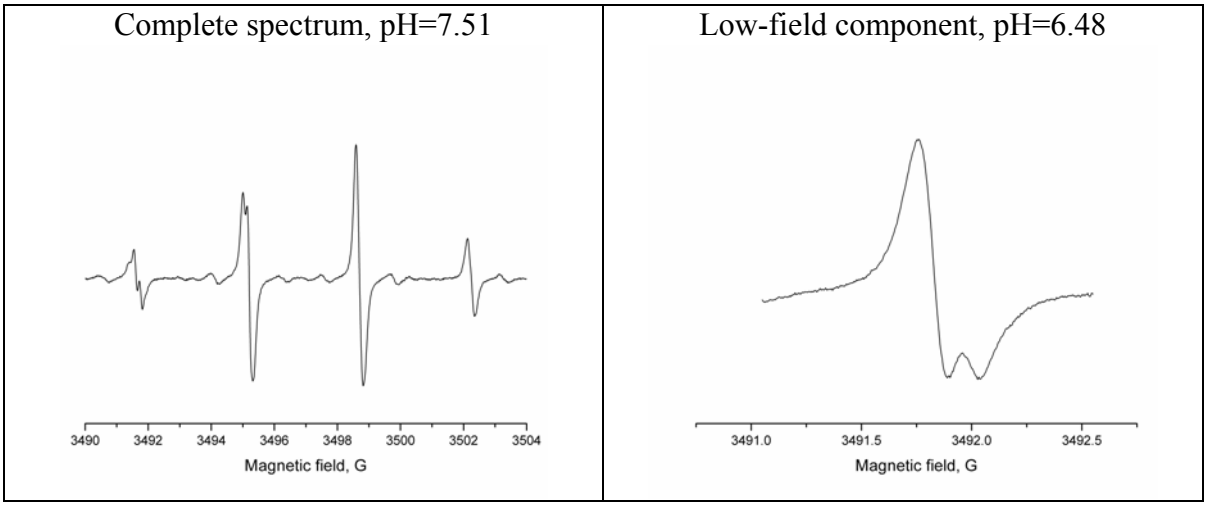


Figure S28: Theoretical fractional composition of AH_3^{3-}, AH_2^{4-}, AH^{5-}, A^{6-} ionic species with respect to the pH



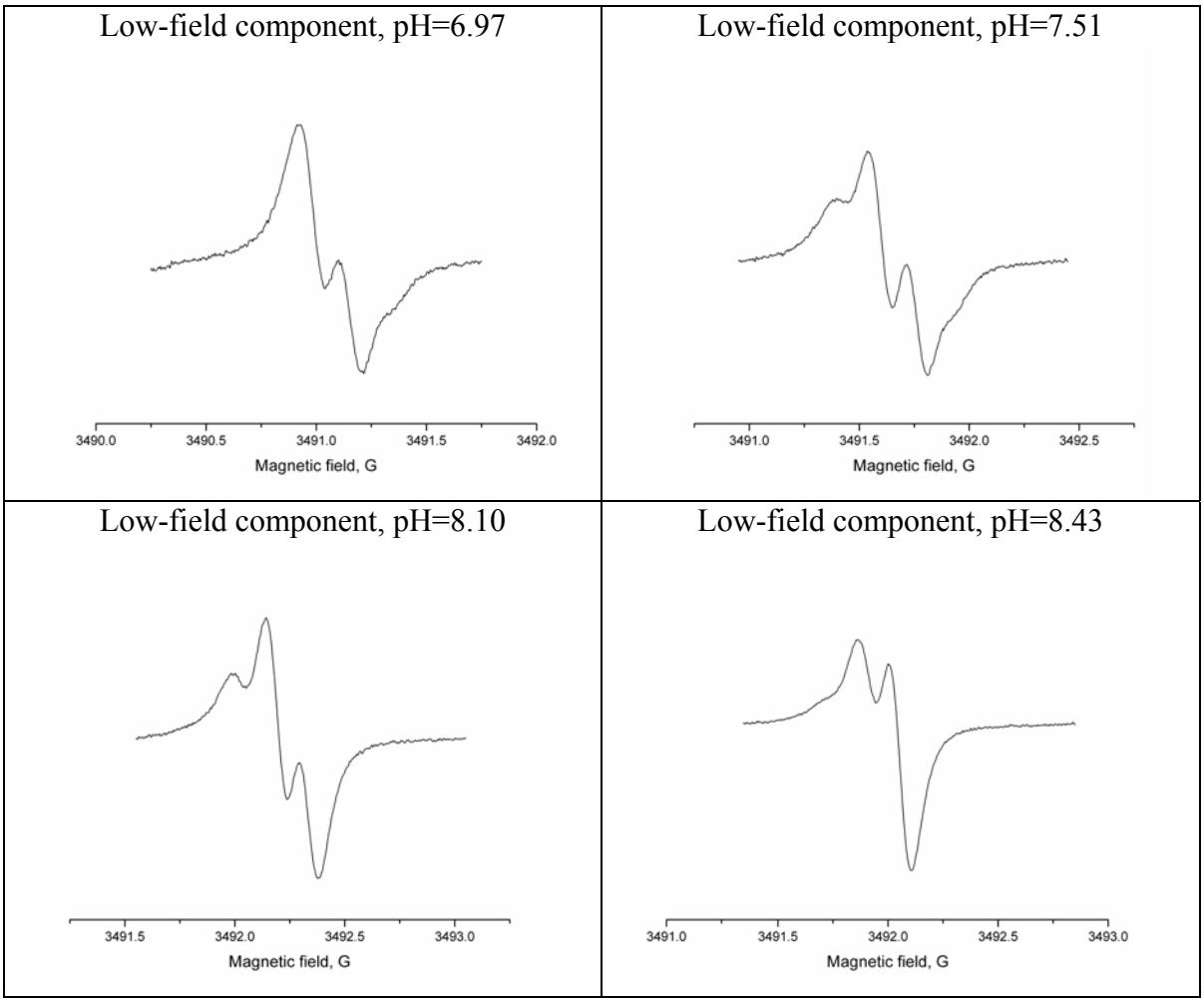


Figure S29: X-band EPR spectrum of **3a** in PB 2 mM at pH 7.51 and the low-field component of the quartet at various pH values.

Acquisition settings were (a) for the complete spectrum: power, 0.5 mW; sweep width, 20 G; modulation frequency, 10 kHz; modulation amplitude, 0.02 G; time constant, 20.48 ms; conversion time, 20.48 ms; sweep time, 41.94 sec; number of points, 2048; number of scans, 5; room temperature; normal air. (b) For the low-field component: power, 0.5 mW; sweep width, 1.5 G; modulation frequency, 10 kHz; modulation amplitude, 0.02 G; time constant, 20.48 ms; conversion time, 20.48 ms; sweep time, 10.49 sec; number of points, 512; number of scans, 16; room temperature; normal air.

3.4 g-factor measurements

The g-factors of **3a** at pH=0.11, 4.98 and 10.50 were measured relatively to TEMPOL (g= 2.00590)[1] used as internal standard. The g-factors were measured in PB 2 mM at X-band.

pH	Ionic form	g (±0.0001)
10.50	A^{6-}	2.00273
4.98	AH_3^{3-}	2.00302
0.11	AH_6	2.00286

Table 1

Acquisition settings were : power, 2.5 mW; sweep width, 80 G; modulation frequency, 10 kHz; modulation amplitude, 0.03 G; time constant, 20.48 ms; conversion time, 20.48 ms; sweep time, 83.89 sec; number of points, 4096; number of scans, 3; room temperature; normal air.

3.5 Influence of ionic strength

The X-band EPR spectra of **3a** 100 µM were recorded in PB 10 mM with 0 mM, 70 mM and 138 mM of NaCl at room temperature under normal air condition. The apparent phosphorus hyperfine splitting constant (a_P) was found to be dependent of the ionic strength (Table 2). This is the consequence of a modification of the pKa associated with the phophonic acids when ionic strength varies. This results in a modification of the fractional composition of ionic species for a given pH and thus a modification of the apparent hyperfine splitting constant. The effect is similar to that observed for the ^{31}PNMR chemical shift dependence of inorganic phosphate with ionic strength.

Solvent	a_P (G)	$3a_P$ (G)
PB 10 mM, pH=7.51, NaCl 0 mM	3.508	10.50
PB 10 mM, pH=7.51, NaCl 70 mM	3.481	10.43
PB 10 mM, pH=7.51, NaCl 138 mM	3.462	10.36

Table 2

[1] *Phys. Rev. B*, **2005**, 72, 085307

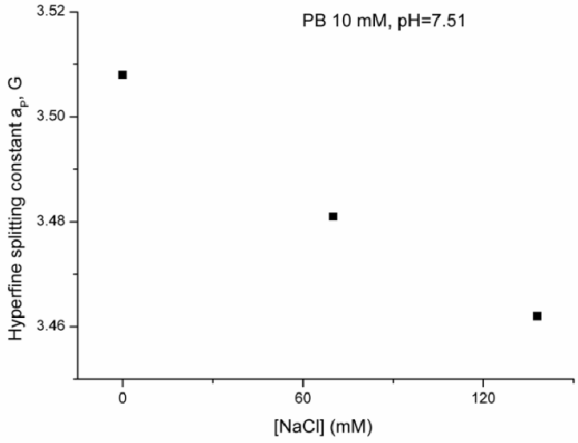


Figure S30: Influence of ionic strength

Acquisition settings were: power, 0.5 mW; sweep width, 20 G; modulation frequency, 10 kHz; modulation amplitude, 0.02 G; time constant, 20.48 ms; conversion time, 20.48 ms; sweep time, 41.94 sec; number of points, 2048; number of scans, 3.